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The Excystment of Trichinella larvae in Artificial Gastric Juice.

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By feeding infected flesh to experimental animals, and examining the stomach contents of the latter at varying periods after feeding, various workers have shown that Trichinella larvae are released from their cysts within one or two hours after reaching the stomach of the experimental host. (Bugge, 1934, Heller, 1933, 1934, Kreis, 1937.) The actual release of the larvae from the cysts has not been followed, though Kreis (1937) on the basis of the appearance of stained cysts recovered a short time after ingestion, suggests that the larvae are released not only by the action of the gastric juice of the host, but also as a result of secretory activity by the larvae themselves within the cysts.

The readiness with which Trichinella larvae may be obtained by digesting infected flesh in artificial gastric juice suggested the possibility of observing the actual release of the larvae from the cysts.

Materials and Methods.

Cysts were obtained from the flesh of a polar bear which died in the London Zoo in March 1937, the voluntary muscles being very heavily infected with encysted Trichinella larvae. The cysts were dissected free from the surrounding muscle fibres in saline, and placed in 1 cc. of artificial gastric juice (1 per cent. granular pepsin in ·5 per cent. HCl) contained in a glass ring sealed on a slide ; the latter was then placed on the microscope stage, warmed to 37°C., and *camera lucida* drawings made at short intervals as digestion proceeded. One dozen cysts were also placed in 5 cc. of ·5 per cent. HCl without pepsin, and in normal saline respectively, and both lots incubated at 37°C.

Observations.

A brief description of these cysts is necessary, since they present several unusual features (Fig. 1). They are oval in shape, both poles being surrounded by an aggregation of fat globules. The walls of the cysts are partially calcified round one pole only, the other pole showing

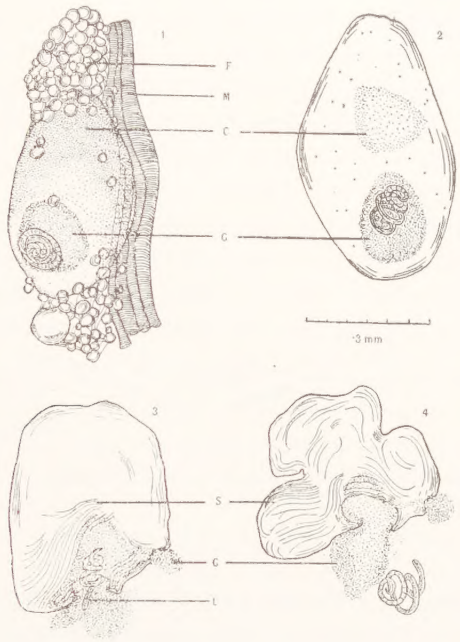
only slight calcification; the walls are also much thickened, and the contained larvae lie near the clear, slightly calcified poles. Each cyst is filled with a clear viscous fluid, while immediately adjacent to the larva is a granular jelly-like mass, the appearance of which suggests a mass of excretory matter released by the larva. The partially calcified walls of the cysts, and the presence of fat globules indicate an infection of relatively long standing, though the larvae were still viable in the majority of cysts.

About five minutes after the cysts are placed in the gastric juice the calcified deposits in the walls dissolve and the fat globules are released from the poles as the areolar tissue holding them together is partially digested (Fig. 2). That the dissolution of the calcified deposits in the cyst walls is due entirely to the action of the acid in the gastric juice alone is obvious from the fact that these deposits are also dissolved away in those cysts placed in the HCl solution without pepsin.

During this period the larvae remain motionless within the cysts, but as digestion proceeds the cyst wall becomes digested at one or more places at the pole where the embryo lies, and the granular jelly-like mass begins slowly to flow out. At this stage, which is reached in about fifteen minutes in the majority of instances, the larva within the cyst begins to show slow movements of the posterior end of the body, these becoming more and more active until the characteristic coiling and uncoiling movement of the larva is evident. As a result of these movements, the posterior end of the larva is inserted through one of the gaps digested in the cyst wall, apparently by chance, and through this the tail of the larva is immediately pushed further. Subsequent excystment is actively accomplished by the larva, which utilises the leverage obtained on the outer wall of the cyst by the hinder end, to pull itself right out of the cyst (Figs. 3, 4). As the contents of the cyst escape into the fluid surrounding it, the walls of the cyst collapse and become characteristically striated in appearance as they are folded and crumpled (Figs. 3, 4). Those portions of the granular mass which are released into the fluid are rapidly dissolved, though in most instances the cyst is not completely emptied.

The average time taken in the examples studied for the larva to be released completely was thirty-five minutes; in about one and a half hours the empty cyst is collapsed to a small mass of fibrous tissue upon

which the gastric juice has no further action. These times are probably quicker than those in the gastric juice of an experimental host, since the percentages of pepsin and HCl in the artificial juice used are higher than those occurring normally in mammals.



Excystment of Trichinella.

Fig. 1. Cyst dissected free, mounted in glycerine jelly.
Fig. 2. Cyst after immersion for five minutes in gastric juice.
Fig. 3. Larva emerging from cyst after fifteen minutes in gastric juice.
Fig. 4. Larva freed from cyst after thirty-five minutes in gastric juice.

List of Abbreviations.

C — Calcified deposits in cyst walls.
F — Fat globules.
G — Granular mass adjacent to larva.
L — Larva
M — Muscle fibres of host tissue.
S — Striations in cyst wall.

It is of interest to note that when digesting larger quantities of muscle to obtain larvae, the latter are released from the cysts before the cysts are freed from the muscular tissue in which they lie. Examination of the sediment from the digestion of muscle in quantity shows empty cysts still attached to the portions of muscle fibres.

The stimuli causing the larvae to move within the cysts appear to be contact of the larvae with fresh fluid and a suitable temperature. Those cysts placed in saline and HCl solution alone showed no change after several hours, except that in the latter the calcification was dissolved. The larvae were still motionless in each instance. Larvae artificially released by cutting open the cysts in gastric juice, HCl, saline and even water at 37°C. almost immediately became active. They were also stimulated to movement within the cysts by pricking the wall at one point with a fine needle, and allowing the outside medium to diffuse in, either the digestive juice, HCl, saline or water. When released into any of these fluids below 30°C. however, the larvae remained inactive.

In a few instances, probably those cysts with thinner walls, it was noticed that digestion proceeded very rapidly, the larva and cyst contents sliding out into the fluid within five to ten minutes ; and in these examples the larvae did not show any activity until they were outside the cyst.

Discussion.

Kreis (1937) in cysts recovered a short time after ingestion and stained, describes a deeply staining granular mass which appears to be flowing from the region of the hinder end of the larva towards the point where the cyst wall is being digested ; and this author suggests that this may be a secretion elaborated by the larva to assist in its release from the cyst. While this suggestion is not readily capable of proof, in the examples watched being excysted it did not seem as if the larva assisted its release in any way other than the purely mechanical leverage described above. In fact, it could clearly be seen in optical section that the cyst wall was attacked on the outside only. The facts that the larva may be released without any apparent activity in a few instances, and that when activity does commence it is not until the cyst wall is broken down and allows the fluid to reach the larva lend support to the suggestion that the cyst wall is digested by the gastric juice alone. While it is probable that the cyst wall before complete calcification is permeable to the fluids in the

muscles of the host, the gastric juice does not appear to stimulate the larva by diffusion through the intact cyst wall but only by contact with the larva after the wall has been broken.

While it is supposed therefore that the larva is released only by enzymic action on the cyst wall, aided by active movement of the larva after the wall has been perforated, it is interesting to note that larvae which have died previously in the cyst are not freed during digestion (Hall & Collins, 1937), and that cysts containing dead larvae may be passed in the faeces of an experimental host after feeding with trichinosed flesh. This latter fact was observed in a ferret which had been infested for experimental purposes by Professor Leiper, the faeces of which examined 24 and 48 hours after feeding showed empty cysts, and one or two cysts which had been perforated without releasing the larvae. These observations support the previous results that the cyst wall is perforated at one or two places only, the remainder remaining intact after long immersion in digestive juices; and it appears probable that the failure of the dead larvae to excyst is due to the fact that they, naturally, did not become active and pull themselves out.

In every instance, the cyst walls are digested at those points immediately adjacent to the larva; at those points, in fact, which in older cysts containing viable larvae, are least calcified; and where, as may be seen in sections, the walls are thinnest. It is possible that the walls of the cysts undergo structural and chemical changes as a result of the deposition of calcium salts which render them resistant to the action of pepsin and intestinal enzymes since the whole cyst wall is not digested. It is known also that certain types of fibrous tissue, such as elastin, are almost indigestible in gastric juice, and other types such as collagen, only slowly dissolved. The ultimate fate of young non-calcified cysts in gastric juice would be of interest in this connection.

Though the cysts used by Kreis were from an infection of short duration, the darkly staining granular mass he describes may be a mass of supposed excretory matter such as that observed in the cysts described above, which also stains deeply with carmine and basic dyes; and in fact, similar masses, though not so well developed, have been observed in sections and pressed mounts of Trichinella cysts from rats and a rabbit in the collection in the London School of Hygiene and Tropical Medicine; infections certainly of a shorter duration than that of the polar bear.

This investigation was carried out while working under a grant from the Medical Research Council to Professor R. T. Leiper, F.R.S., to whom my sincere thanks are due.

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A Comparative Study on Schistosomiasis in the Berber Region of the Anglo-Egyptian Sudan.

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Endemiology.

Although it may be objected that the present paper deals with a limited population in a circumscribed area, the conclusions drawn are in the main applicable to any zone of endemic Bilharzial infestation. The numbers examined form a considerable series, and the distribution and age and seasonal incidence of the contrasting infections are of some interest. A personal relation with those infected has been, and still is, maintained.

For the above reasons, and to allow of detailed study and effective comparison between the disease in its vesical and visceral forms, the following study on a known population under continued personal observation has been submitted.

Population Groups.

The population of the Province is divisible, broadly, into three groups : *a*. Settled Agricultural, *b*. Nomadic, *c*. Industrial and Urban.

The material of this paper is drawn mainly from surveys carried out in the first, and largest, group ; of which the people inhabit ribbon cultivation on either side of the river. The highest density of population is in the neighbourhood of large pump schemes (Fig. 1), of which there are 28 in the Province, four being Government owned. They are grouped also in relation to " Basins "—large tracts of land lying below the level of the maximum flood peak of the Nile, and affording seasonal cultivation. It may be mentioned in passing that the Nile shows two flood " peaks "—one occurring about the middle of August, and the other, less marked, occurring in the first week of September. It will be shown that this rise and fall of the river exerts a direct seasonal influence on the incidence

of the disease, and that the methods of agriculture—particularly of irrigation—affect the local density of infection.

It was not found possible, unfortunately, to determine incidence of Schistosomal infestation among the nomadic group, who live in the "back-lands," and take advantage of seasonal pools, "rain crops" and grazing. (Humphreys, 1932.) From the few encountered it would appear that intensity of infection is high : and it is probable such nomads form a reservoir of infection. Coming as they do in the latter part of the dry season to the fringe of the riverain cultivated areas, they may re-establish infestation in a cleared area. In the absence of reliable information this group is not further discussed. They are not however considered as a menace to the settled population, among whom unfortunately a sufficiently high proportion of carriers exists to ensure perpetuation of infection. Mention is later made to this in considering the problem of elimination of infection.

The industrial and urban group—principally the artizans and officials of the Sudan Railways in Atbara, with the associated traders, present few cases of infection. Infection is not acquired in Atbara, and cases are found either (*a*) to arrive infected, or are (*b*) infected or reinfected when on leave, or on duty in out stations. Only one case of infection in a European (British) official has been found—detected only in the course of routine examination while he was in hospital with malaria. (He himself was unaware of the condition, which must have been contracted while duck-shooting in one of the Southern Provinces.) The case is an interesting example of minimal infection, which had continued over three years without causing the patient any obvious discomfort. The ova passed were found to be non-viable, and no specific treatment was considered necessary.

Irrigation and Topography.

The distribution of Bilharziasis throughout the Province is not uniform. It is considered that the factors principally affecting incidence are, first, the method of irrigation, and second, the rise and fall of the river. Irrigation is carried out by :—

a. Waterwheels (Sagia) drawing either from a well, or direct from the river.

b. By "shaddoof"—a counter-balanced bucket, swinging on a beam.

c. By mechanically driven pump.

The seasonal rise of the river is also used to advantage by the aid of a series of canals and dykes : by which large areas of level low-lying land—the basins formerly mentioned—are temporarily flood covered. Such basins, though presenting a serious sanitary problem (from the point of malaria) do not appear greatly to affect the Bilharzial incidence.

In the course of this investigation it has been found that irrigation by waterwheel, or by " shaddoof," does not tend to establish a focus of infection ; but that the reverse is the case with pump fed cultivation. Leiper (1935) stresses the effect of change from basin to perennial irrigation, and consequent increase in human Bilharzial infection from 10 to 90 per cent. (in the Delta)—the direct result of the great increase in the snail population. Water drawn from the river by man-power, or by cattle driven wheels, is carefully husbanded, and strictly doled out in such manner that effective drying takes place between waterings, and is run through narrow channels giving a rapid flow : when supplied by pump, however, the native cultivator " over-waters " the crop, and if not strictly supervised, uses the secondary and tertiary canals, in which flow is sluggish, for all his domestic offices—thereby setting up a cycle of infection. Snails abound in the canals of pump schemes, but I have not yet found them in the sagia-ditches. (Stiven, 1931.)

Under optimal conditions breeding of snails may take place throughout the year. Humphreys (1932) found that snails were established in irrigation canals 18 months after their opening and had spread throughout the scheme in 30 months.

There is no question but that snails are drawn into canals through the pumps ; that is, canals even if treated, are promptly re-infested on occurrence of the first watering. I have found that in many areas, particularly in the cataracts north of Atbara, the silt may be composed of myriads of miniature snails. On no account should pumps draw other than from the free running stream.

In addition to the above noted primary factors, local topography may vary the incidence. The characters of river and river bank vary not only from district to district, but with the time of year. Where there are wide shallow rocky stretches with numerous pools, where there are grassy banks with weed-covered pools, and above all, where long shallow creeks (" Kheiran ") run some distance inland, there one will find a locally high

incidence in Bilharzial—and malarial—infections. It has been suggested (Stiven, 1931), and confirmed locally, that the incidence of *Schistosomiasis mansoni* is greatest about end-canals of pump-fed irrigation systems. (Fig. 4.)

For convenience in reference, six " survey zones " north and six such south of Atbara have been marked out (Fig. 1). The principal Surveys referred to in this paper were carried out by the author in zones 1, 2, 3 *north* of Atbara, and in 1 and 2 *south* of Atbara.

Seasonal Variation and Incidence.

The seasonal incidence of the disease is of interest, marked in vesical (*S. haematobium*) infection, it is much less evident in visceral (*S. mansoni*) infection. This is related, in all probability, to seasonal exacerbation from fresh repeated infection with the more common *S. haematobium* ; and possibly to drop in individual resistance following on continued labouring in the fields, to concurrent malarial infection, and in some instances, to malnutrition. During the fall of the river the number of cases coming up for treatment gradually increases (Fig. 3) and the greatest number present during the months of March, April and May. It may be, however, that records are appreciably affected by the demand for agricultural labour in preceding months, when the cultivator has no time to attend at his local dispensary. Others have drawn attention to this seasonal variation. Archibald (1933) is of opinion that " infection is acquired during the months of the year when water is sufficiently shallow to permit of a high concentration of cercaria. In the case of inland lakes and water courses, the seasonal incidence is from October to the end of January ; in the Nile backwaters, the danger period is usually February to June." In the Province the rise is attributed to the pools and stagnant creeks left by the falling river. Figures show that it is in villages in the immediate neighbourhood of such that the most sudden and marked increase in infection occurs.

Intermediate Hosts.

The snails most frequently found may be grouped under the following genera, *Bulinus, Cleopatra, Melanoides, Planorbis* and *Bythinia*. *Bulinus* spp. and *Melanoides* spp. are widespread throughout pump schemes and *Cleopatra* spp. may be found almost anywhere in the Province, both north and south of Atbara. *Planorbis* is relatively uncommon, but has

been noted in certain of the pump schemes. It is, however, noteworthy that *Planorbis* spp. have not been detected in any number in relation to the zones of visceral infection : and that in these areas *Bulinus* spp. and *Melanoides* spp. are the common varieties met with. Of the two, *Melanoides* spp. (*M. tuberculata*) is considered the probable host. Gopsill (1929 & 1931) in reporting on Schistosomiasis in Nyasaland, gives *M. tuberculata* as an intermediate host of *S. haematobium*, and of *S. mansoni*. Archibald (1933) on laboratory investigation, found that in the Sudan " *Bulinus truncatus* (including the species *B. contortus*, *B. dybowskii*, *B. innesi*) is the intermediate host of *S. haematobium*, and *Planorbis boissyi*, *P. pfeifferi*, *P. alexandrinus* and *P. herbeni* the intermediate hosts of *S. mansoni*." Van den Berghe (1934a) also implicates *Melanoides* as harbouring schistosome cercariae. *M. nodicunata* has been shown by Dye (see Zavattari, 1929) to act as the intermediate host of *S. haematobium* and Zavattari (1929) is of opinion that *M. tuberculata* may act as an intermediate host. (Baylis (1931) gives a list of corrected names of vectors of human Schistosomiasis, *Melanoides* spp. not being included ; it is not claimed that the list embraces all vectors.)

I have obtained both furcocercous and non-furcocercous cercariae from *Melanoides* spp. and double infection of the snail has been noted : *Cleopatra* spp., common throughout the Province, also have been noted as infected with furcocercous cercariae, and with non-furcocercous of two types. The non-furcocercous are broadly divisible as pigmented and non-pigmented—the pigment being disposed anteriorly in the form of " eye spots." The furcocercous cercariae are invariably non-pigmented. Snails of *Planorbis* and *Bythinia* spp, have also been found to harbour furcocercous cercariae. It is not, of course, suggested that the furcocercous cercariae noted are necessarily those of *S. haematobium* or of *S. mansoni*, although presumptive evidence is strongly suggestive : and it is greatly regretted that pressure of routine work did not allow of detailed investigation of the morphology of the cercariae noted. Reference must be made to the studies of Archibald & Marshall (1932a, b, c) on the morphology of the cercariae of *S. haematobium* and *S. mansoni*, and on unclassified cercariae obtained from molluscs in the Sudan. Emphasis is laid on Leiper's postulate that " the identification of any species of cercaria is only practically possible by recovering the adult worm from experimentally infected animals." (Further observations on the

anatomical structure and identification of cercariae may be found under Archibald & Marshall, 1932d, Blackie, 1932 and Bettencourt & Da Silva, 1928.)

As noted above, many snails show evidence of infection not necessarily of Bilharzial type—being intermediate hosts to trematodes ultimately parasitic upon animals, birds or fish. Extensive investigation of the gut and other viscera of cattle, sheep and goats butchered in Atbara failed, however, to show evidence of local infection of animals. Archibald (1933) states that bovine Schistosomiasis (*S. bovis*) is fairly prevalent in the Sudan; and numerous schistosome infections of cattle, sheep and goats are described in the literature. Veglia & Leroux (1929) have described a sheep schistosome with terminal spined eggs and have named it *S. mattheei*. Goats, cattle and water buffaloes in India are found naturally infected with *S. spindale* (Archibald, 1933). *S. bovis* is noted (MacHattie & Chadwick, 1932 & 1933) as parasitic in horses, donkeys and mules in Irak, and infections are essentially portal and intestinal : and it is further stated that " *S. mattheei* as described by Veglia & Leroux (1929) is indistinguishable from *S. bovis* and the latter occurs in Irak." Van den Berghe (1934a) has noted *S. bovis* and *S. mattheei* in sheep, oxen and man.

Possibility of Aberrant Infection.

The possibility of aberrant animal infection in man is of great interest, and was fully considered in the course of surveys detailed in this paper, but has never been noted, and Archibald (loc. cit.), in a much wider enquiry, has not found the " characteristic ova of *S. bovis* . . . in the faeces or urine of natives of the Sudan." Blackie (1932) implicates *S. mattheei* as a parasite of man, and claims to have found this parasite in ten men : in eight of these diagnosis was made on the egg form, in two, the adults were found post-mortem. In the latter, *S. haematobium* was also present. He is of opinion that *S. mattheei*, in Southern Rhodesia, is not a negligible parasite of man. But reliable diagnosis cannot be made on egg forms and measurements, as such may show—and in material gathered on Survey did show—marked variation even in the same individual. In support of this, reference may again be made to the findings of MacHattie and Chadwick (1932–3.) (loc. cit.) who have shown that a diseased *S. bovis* may produce eggs corresponding in shape and size to those of *S. haematobium*. The egg illustrated by Blackie as that of *S. mattheei* is

indistinguishable from that of *S. bovis*. They illustrate consecutive eggs from a uterus, one *S. bovis*-shaped, the other *S. haematobium*-shaped, and immature eggs of *S. haematobium* from human urine showing elongation and spindle shape.

" About 1 per cent. of females show many typically shaped *S. bovis* eggs in utero and in the same uterus one or more typically shaped *S. haematobium* eggs having the same measurements as the egg of this parasite in the human subject . . . ." " Approximately 0·2 per cent. of females contain solely eggs of *S. haematobium* shape and measurement. Such females are only distinguishable from this parasite in man in that the vitellaria occupy approximately one half of the total length of the female." (MacHattie & Chadwick, 1932–3.)

MacHattie and Chadwick are of opinion " that Blackie has failed to produce any convincing evidence in support of his statement incriminating cattle and sheep as constituting a potential reservoir of human urinary Schistosomiasis."

Spurious parasitism with *S. mattheei* is noted by Blackie (in the same Survey as that implicating man as a host for this schistosome), ova being noted in a case where a half raw ox-gut had been consumed on the previous day.

Fisher (1934) in the study of intestinal Schistosomiasis in the Stanleyville District of the Belgian Congo holds that the schistosome implicated forms a new species, *Schistosoma intercalatum*. But " none of the morphological characteristics of this parasite, apart from the ovum, are such as to enable it to be differentiated clearly from either *S. haematobium* or *S. bovis*."

As pointed out above, however, neither size nor shape—in which great variation has been recorded in the present Survey—are reliable criteria, and of themselves do not justify the labelling of a new species. One might as well classify farmyard fowls in the shape, size and colour of their eggs ! That the lesions produced were located between anus and pelvi-rectal junction is of interest, but terminal spine ova in and from such areas have not uncommonly been noted in *S. haematobium* infections. Moreover, Schwetz & Baumann (1930) found that in the Stanleyville district *S. haematobium* is more often the cause of intestinal infection than is *S. mansoni*. Raynal (1929) has collected references as to aberrant localisations for schistosomes in man : ova of *S. haematobium* have

been found in stools, alone and in conjunction with those of *S. mansoni* : and in the urine co-incidentally ova of *S. mansoni* have been found, both singly and in conjunction with those of *S. haematobium* in the stool. (Brumpt (1930) has collected the literature of cases showing ova of *S. mansoni* in urine, and ova of *S. haematobium* in the faeces.)

The author has seen only two cases in which ova of *S. haematobium* and of *S. mansoni* occurred together in the faeces. Both cases were young males. No instance of double-infection has yet been found in children.

In spite of co-existent *S. haematobium* and *S. mansoni* infections within one agricultural scheme in this Province double infection has not been noted. Yet only a few kilometres distance intervened between villages showing, in one instance vesical and in the other intestinal infection. Even among the children (commonly the principal group in vesical infection) in the known zone of rectal infection, no case has yet been found to show co-incident urinary and faecal infection.

Chemical Reaction in Relation to Environment of Intermediate Hosts.

It is suggested that this quite peculiar feature is due to variation in snail hosts, and this variation in turn to the chemical reaction of the water irrigating the affected zone. The chemical characteristics, it is considered, may render the water suitable only for snails of certain species, thereby limiting the spread and propagation of the optimum intermediate host ; and may even affect the motility and penetrative powers of miracidia and cercariae alike. In point of fact, the water of adjacent irrigation zones has been found to vary in *p*H., and in the laboratory I have found that snails are very susceptible to the reaction and oxygen content of water. Alkaline waters may harbour *Bulinus*, but acid waters do not favour its development (Gauthier, 1934).

Hassan (1933) has found miracidia of *S. haematobium* and *S. mansoni* very susceptible to alteration in *p*H. The following percentages of chlorides killed in 4 hours : Na, 0·9 per cent. ; K,Mg., (NH_3), 0·7 per cent.; Ca., 0·5 per cent. The relation between reaction of water and development of intermediate host and parasite has not sufficiently been studied ; and it is much regretted that no facilities were here available for a more detailed study than that undertaken.

Optimum Habitat of Intermediate Hosts.

Snails are found most abundantly in the weeds and mud fringing secondary and tertiary canals. Temperature affects the rate of breeding but breeding takes place throughout the year. The spread of snails is effected in the main by irrigation, though it is difficult to account for the infestation of scattered seasonal pools. Carriage of snails and eggs may be effected by birds, as suggested by Humphreys (1932). The eggs are found on floating and submerged vegetation, which also affords harbourage for snails, as does the brickwork of bridges and "escapes" and posts or stakes in the canal. Palm fronds immersed in the canals have been used as traps. The canals may be cleared of snails by (*a*) drying and re-excavation, (*b*) treatment of the water in partially emptied canals with Sizoline or Ialine (Commercial Carbolic Acids). Both methods are here in use.

Reinfestation of cleared irrigation zones takes place from the river, via pump and main canal. The "trapping" of canals has conclusively shown that snails are constantly thrown into the main canal during pumping and that they may be found settled in mud and on vegetation even at one kilometre above intake. Snail concentration becomes progressively higher as one approaches the terminal stretches of secondary and tertiary canals; and breeding is most marked in the sluggish water of these terminal ditches.

Leiper, cited by Khalil (1930) has suggested that "further investigations are required to determine whether there is not an upward migration from the smaller to the larger canals at certain times of the year." In so far as this Province is concerned it is positively affirmed that no such migration takes place; and that, though in certain circumstances snails will float up to, and actively move on, the surface layer of the water, the probability is against migration in any area. As noted above, the influx of snails is from the river and main canal, though conditions for multiplication are optimal in the terminal canals. A sufficient distinction is not always drawn between *infestation* of river and canals with snails and *infection* of these localities with Bilharzia.

The main sources of infection of man are the small canals and drains, in which snails abound. They are found in great numbers in the swirl pits at the proximal part of the tertiary canals. Urination and defaecation into these pits and the supply canals is of frequent occurrence, in spite of

prohibition. The pits ultimately contain stagnant water which has to be bailed out (by hand) as an antimalarial measure, and this completes the infective cycle.

The free running water of the river is not a source of infection, but infection may occur (as previously noted) where the falling river leaves numerous stagnant pools among the grassy hollows of the bank. Snails swarm in the mud and on the vegetation of such pools. During low river, snails and their " eggs " may be collected from the main stream, especially where sluggish, by the use of traps of brush-wood, or palm fronds. For this reason, sanitary approval is given only to irrigation schemes in which the pump draws from the main stream.

Sagia (waterwheel) irrigation is comparatively free from danger, as snails do not swarm in the sagia pits or wells, and will not withstand the sudden and repeated drying of the narrow quick flowing sagia ditches.

The free-flowing river though the source of the intermediate host is not a source of Bilharzial infection.

Jane (1932) has reported on the distribution in a major pump scheme and during the investigation was associated with the writer, who has repeated his observations and entirely agrees with his conclusions. It is considered that but few snails survive the drying out of canals, and that they play little or no part in subsequent re-infestation of new flooded canals. Snail " eggs " will not withstand drying, even for a brief period.

It has been suggested by Gopsill (1929–1931) Jane, (loc. cit.) and others that snails may survive the dry period (of winter closure of canals) by burrowing into the soil while it is yet moist. Gopsill has found *Melanoides tuberculata* at a depth of 5 feet in the mud of a dried river bed : but it is considered that such findings can be explained by silting, and by the cracking of drying canals and stream beds, the snails then following the gradual subsidence of water into these cracks. It has not proved possible to corroborate these findings locally, in spite of repeated and extensive examination of dried-out canal beds. Although a few snails are found buried in ooze at depths of two to three inches, it is obvious that they have been covered by silt and no evidence of " burrowing " has been substantiated. Excavation to a depth of two feet also failed to produce live snails.

Barlow (1933) found that in Egypt *Planorbis boissyi* and *Bulinus contortus* survive the dry period by production of an epiphragm of dried slime. He obtained live, but non-infected, snails from a canal bed which had been dry for 30 days. (The production of this epiphragm in non-operculated snails has been the subject of recent interesting articles in the Illustrated London News, 1935.)

Surveys.

The surveys on which this paper is based were carried out in February and March of 1933 and 1934, in areas Nos. 1, 2, 3 North, and 1, 2 South (as indicated on Fig. 1).

In the northern zones 1,083 persons were examined and 69 (6·4 per cent.) were found infected with *S. haematobium*. No infection with *S. mansoni* was at that time noted. (Two cases of intestinal Bilharzia have since been found, but in each case infection was found to have occurred during work in the Abu Selim area.) In the Southern zones 1 and 2, 1,507 persons (all males) were examined, and 19 (1·3 per cent.) were found to have urinary (*S. haematobium*) infection.

In zones 1 and 2 endemic intestinal infection was found. Attention had first been attracted to certain villages in these areas (Abu Selim, El Ogar, Umerab and Timerab, in zones S1, and Rau, Fereikh and Aliab in zone S2). In zone S1, 310 persons were examined and 109 (35·1 per cent.) were found to show the ova of *S. mansoni* in their stools. In zone S2, 167 were examined, and in 93 (55 per cent.) the ova of *S. mansoni* were demonstrable. Distribution of infection throughout the villages is by no means uniform and is most marked in such as are related to subsidiary and end-canals. (Attention has already been drawn to the snail population of such canals.) It is of interest to note that the village of Hassabalab, which is not in intimate relation to any end-canal showed no infections among 70 persons (males) examined : and a further point of great interest is that no cases of *S. haematobium* infection were detected among children in zone S1.

Incidence of the Disease : and Clinical Manifestations.

Analyses of the cases of urinary Bilharzia showed by far the highest incidence of infection to occur in villages adjacent to narrow creeks, and collections of stagnant water. Incidence varied from 1·6 per cent. to 50 per cent. of persons examined.* Sagia (waterwheel) cultivation had

* According to Village.

no relation to incidence. According to area from 85·7 per cent. to 92 per cent. of infected persons were under 21 years of age and the highest incidence of urinary Bilharzia occurred in the " 5 to 15 years " age group. In brief, urinary Bilharziasis is primarily a disease of children. Few cases were found over 25 years of age. Similar findings are later recorded by Dixon (1934) (in Katanga) who found the highest incidence between the ages of 10 and 15 years, and no infections among persons over 45. He ascribes this to the comparatively rapid production of immunity. In this, however, I am not disposed entirely to concur, holding that persistence of infection—and the grave complications found in higher endemic areas, such as the Nile Delta—are dependent on the initial intensity of infection, and on repeated infections. Christopherson (1930) has drawn attention to the danger of neglecting initial infection in the child, and to the results of added infection in adult life.

No gross lesions have here been noted as following on infection with *S. haematobium*. Blackie (1932) noted in the course of Survey in Southern Rhodesia that gross clinical manifestations were rarely associated with (the local) urinary Bilharziasis ; but gives a warning, in that 12 post-mortem examinations showed a greater degree of tissue injury than clinical examination had suggested. He was, however, classing infections according to the egg count in the urine : and the numbers noted cannot be accepted as a guide to the actual worm load. Pijper (1934) is of opinion that in most adults the damage amounts to little more than periodic loss of blood, but that in children infection may have serious results. Incidentally, he notes that most local cases occur in the young.

A sharp contrast, both in age incidence and clinical finding, is given by infection with *S. mansoni*.

This may affect all members of the population, incidence rises with age, and the resultant lesions are both extensive and severe, and especially involve liver and spleen. Here again, intensity of infection is considered the decisive factor in the production of the ultimate picture. Some 15 per cent. of persons examined present no obvious feature, and can only be detected on survey. These cases may complain only of slight and infrequent attacks of diarrhoea—for blood may never be obvious in the stools. Gopsill (1932) has stressed the value of the history of obvious dysentery of 7 days duration, even some years back, as indicative of the

onset of Bilharzial infection. Such history is here of no value, dysentery being of common occurrence among cultivators.

All cases cannot be diagnosed on purely clinical grounds. The number of eggs noted in a faecal smear bears no direct relation to the severity of symptoms, and in about 15 per cent. of cases were present without clinical manifestation.

Splenic enlargement (in zones S1 and S2) is of common occurrence. Nor can this splenomegaly be ascribed solely to coincident malarial infection. It is not the invariable rule ; but the average history suggests that all cases ultimately show it, though infection may persist for months or years before enlargement occurs. The incidence and degree of splenomegaly varies greatly according to locality. In zone S2, 35 per cent. of infected persons show splenomegaly in contrast to 75 per cent. in the Abu Selim area, and individually the increase is less extreme.

Methods of Examination.

In investigation of *S. mansoni* infections the method of examination was by thick faecal smear. The portion of stool was emulsified with water, and such quantity placed on the slide as to give a " ground glass " appearance when the coverslip was added. No stool was considered as " presumably negative " until three separate portions had been emulsified and examined. Owing to lack of staff and time, concentrative methods could not be applied : and it is, of course, obvious that the method adopted cannot have displayed all infections—it is sufficiently amazing that the ova of 60 per cent. of cases were detected on initial examination, and that in one village (Abu Selim) 67·3 per cent. of persons examined were found infected !

In the investigation of *S. haematobium* infections, the urine of all cases was tested for albumen. If albumen, or obvious blood, was present the urine was then rapidly centrifuged and the deposit examined microscopically. In *all* cases in which blood was present in the urine ova were found, though not necessarily on the first examination. 25 adults and 7 children (all males) showed albuminuria only, without blood or ova. Van den Berghe (1934b) draws attention to the occurrence of ova without coincident albuminuria (in 22 out of 73 cases) ; and to the occurrence of albumen, pus and blood in 11 cases, in the absence of ova. While the latter is not improbable—and I have noted its occurrence—I have yet to

find a case in which the passage of ova was not associated with albuminuria. Ramsay (1932) has elaborated an equation to evaluate the significance of abnormal constituents in the urine in areas of endemic *S. haematobium* infection, and to show that the "Coefficient of Association" is high (0·93), and that haematuria is an index of infection :

$$C = \frac{(AB)\,(ab) - (Ab)\,(aB)}{(AB)\,(ab) + (Ab)\,(aB)}$$

A = ova present a = ova absent C = coefficient.
B = blood present b = blood absent

The figures given for urinary Bilharziasis relate exclusively to males. It is alleged that the incidence among women and girls is low. (The figure of 0·85 per cent. infection among females as against 11·5 per cent. among males is quoted in the Annual Report (1932) of the Director, Sudan Medical Service.) I have, however, no comparable figures for this Province : but admissions to hospital suggest that the ratio is rather higher.

It must not be forgotten that a very large proportion of *S. haematobium* infections are latent, or relatively without symptoms. It has been suggested (Tsykalas & Riegl, 1929) that such latent infection may be made evident by injection of Emetine (0·059–0·19) with subsequent re-examination of the urine some five hours later. I am doubtful of the value of this procedure, and no occasion has yet occurred on which it could be usefully employed.

Sanitary Control.

As a measure for the suppression, or even control, of the disease it is obvious that treatment of the individual alone is comparatively futile. Attention has already been drawn to the relatively high proportion of the infected in the population who do not complain of the disease, and who are not accessible to treatment.

Measures must be directed against :—

1. Fouling of water by infected persons.
2. Spread of the intermediate host.

The first essential is the provision of wells. Until such time as villages are provided with an adequate supply of well water, and the use of

water from irrigation channels is prohibited, no diminution in incidence need be expected.

At present, the treated and presumably cured individual is only too frequently re-infected almost immediately on return to his native village.

The second essential is the provision of some effective form of latrine. In the dry and sandy soil of this Province it should be possible to devise some form of trench latrine from purely local materials. The difficulty is to enforce its use : and local custom at present contributes both to spread and persistence of infection. It is hoped, in time, to familiarise the people with the dangers attendant on bathing, washing and defaecating in irrigation ditches. Great importance is attached to persuasive propaganda, and encouraging response has already been obtained.

Energetic measures are required against the intermediate hosts, and the following recommendations are put forward :—

1. All canals and ditches to be cleared of weed to one foot below low water level.
2. Canals to be dried off when possible, and to be treated with Ialine, or Sizoline, prior to complete drying.
3. Pools near sluices and bridges should be so treated every 30 days, or preferably baled out. Subsidiary canals should be baled out at " scour " within 3 days of watering, or treated with fuel oil.
4. All stakes, branches and brushwood should be cleared from canals.
5. Aprons on either side of bridge regulators should be carried beyond foundation width, and the brickwork rendered with cement.

Snails are carried into subsidiary (tertiary) canals in great numbers, and the use of palm fronds, implanted at points 10ft., 20ft. and 30ft. distant from the inlet has greatly assisted,

1. In reducing the numbers of snails escaping to the lower and more stagnant reaches of subsidiary canals.
2. In investigation into the predominant snail types, and the incidence of cercarial infestation of such snails.
3. In determining rate of spread of snails throughout canals.

Success in the elimination of Schistosomiasis will only be achieved by elimination of the intermediate host : in short the key to the problem as a whole, is *sanitation, not medication.*

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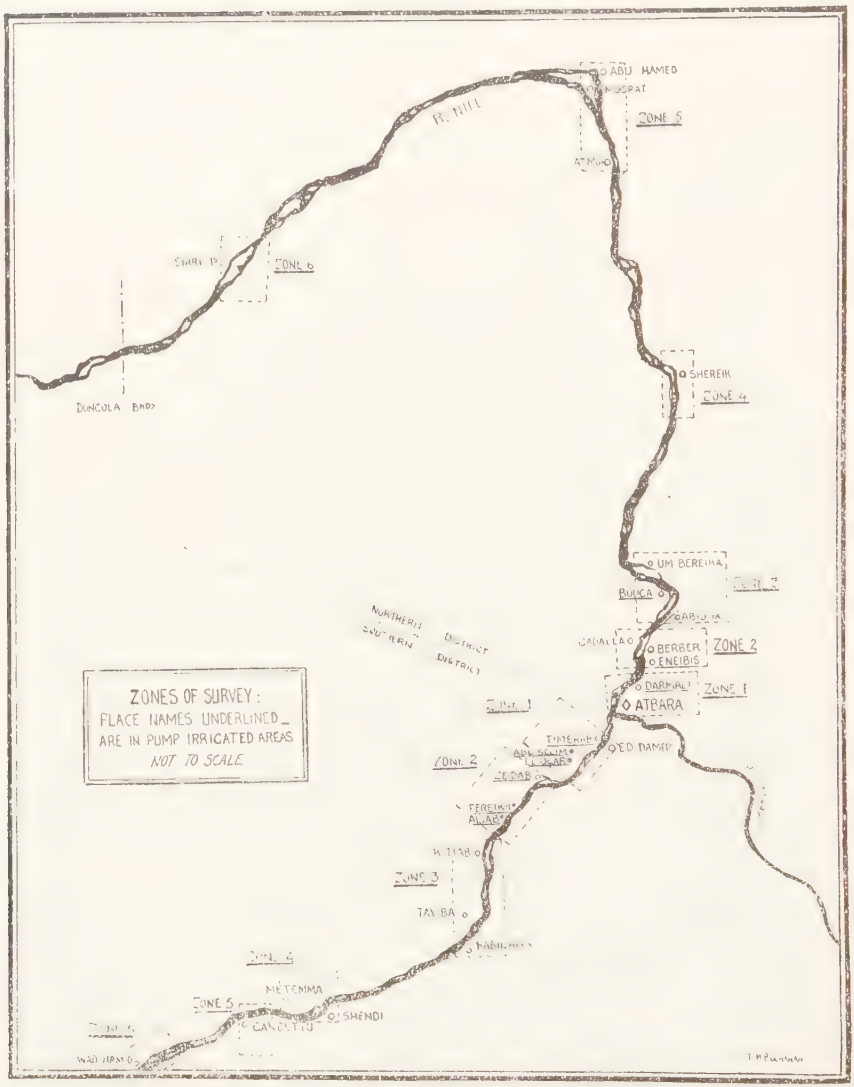
ABU HAMED
R. NILE
ZONE 5
ATBARA
ZONE 6
DONGOLA
SHEREIK
ZONE 4
UM BEREIKA
BUIKA
BERBER
ZONE 2
ZONE 1
ATBARA
ZONE 2
ZONE 3
TAY BA
MIETENMA
SHENDI
ZONES OF SURVEY:
PLACE NAMES UNDERLINED
ARE IN PUMP IRRIGATED AREAS
NOT TO SCALE

Fig. 1.

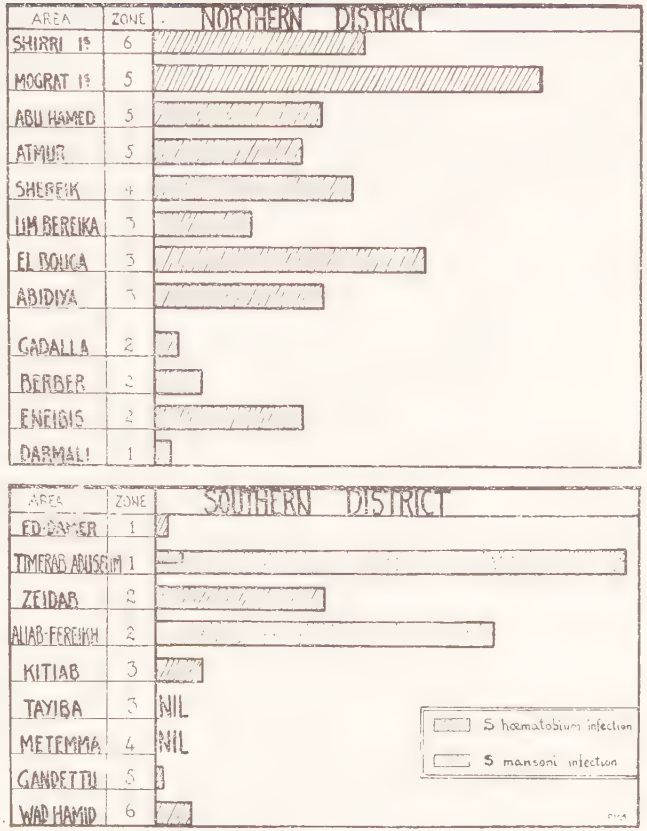

Fig. 2. Index of incidence of Schistosomiasis based on Dispensary returns.

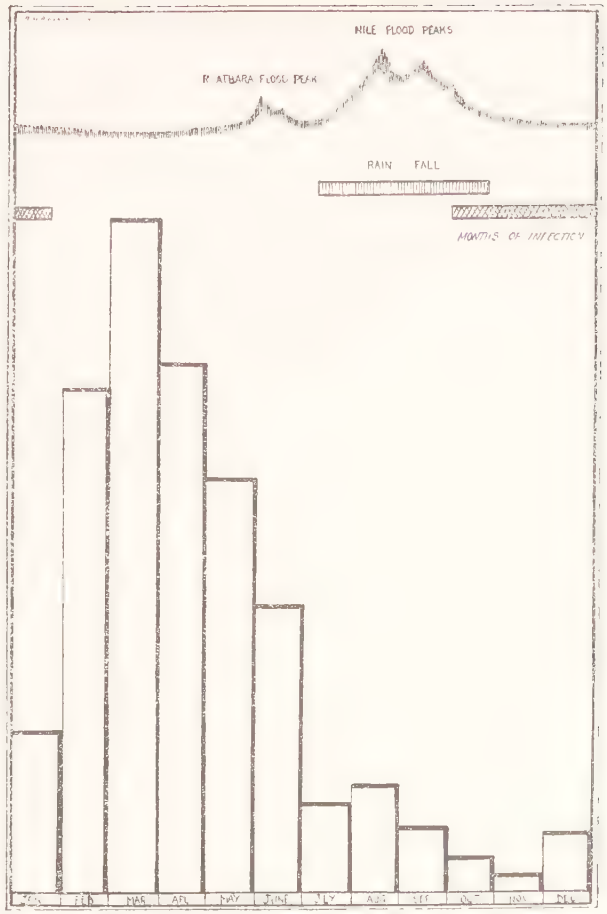

Fig. 3. Seasonal incidence in relation to floods and rainfall.

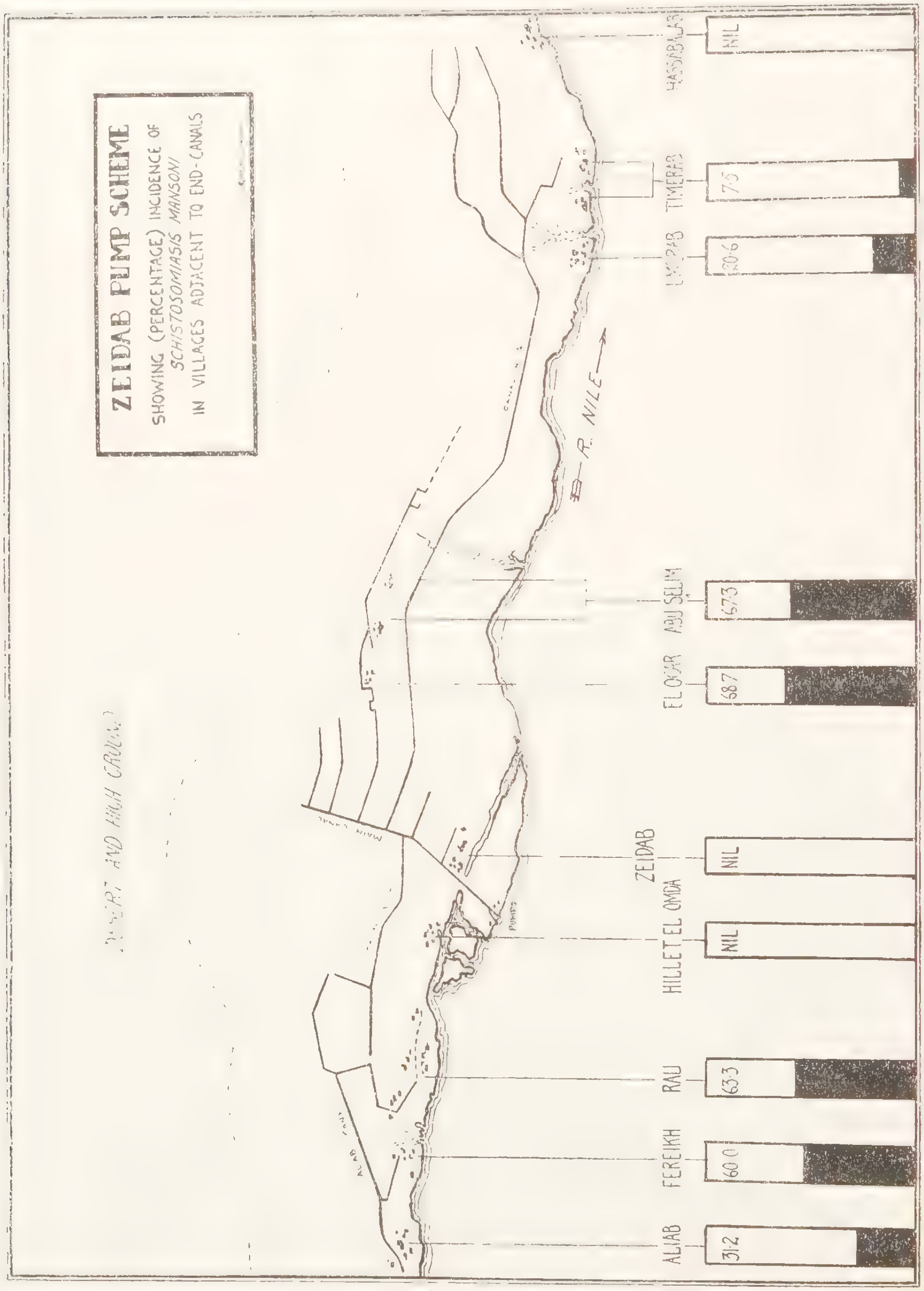
ZEIDAB PUMP SCHEME
SHOWING (PERCENTAGE) INCIDENCE OF SCHISTOSOMIASIS MANSONI IN VILLAGES ADJACENT TO END-CANALS
R. NILE
NIL
TIMERAB
7.5
30.6
ABU SELIM
67.3
68.7
ZEIDAB
NIL
HILLET EL OMDA
NIL
RAU
63.3
FEREIKH
60.0
ALIAB
31.2

Fig. 4.

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Observations on the susceptibility of certain varieties of oats to "tulip-root" caused by the stem eelworm, *Anguillulina dipsaci.*

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Introduction.

"Tulip-Root" in oats caused by the stem eelworm, *Anguillulina dipsaci*, is of widespread occurrence in Great Britain, annually effecting much damage to oat crops which results in diminished yields of grain. At this Institute the disease has been under investigation for some years and during the seasons 1935, '36 and '37 tests have been carried out on the susceptibility to it of a number of different oat varieties. An outcome of some practical importance has resulted from this work in that it has been found that two varieties of winter oats have, for three seasons, proved resistant to attack though grown under the same conditions and subject to the same risk of invasion by the parasite as other varieties which have proved susceptible to attack. These two varieties have consistently manifested no symptoms of disease during the three seasons and on microscopic examination have been found to harbour very few examples of the parasite whereas susceptible varieties have shown the characteristic symptoms of disease in stunted growth, greatly swollen discoloured leaf-bases and their tissues have harboured large numbers of the parasite. The purpose of the present paper is to put these facts on record and to give some account of the investigations out of which they have arisen.

Experimental.

Before dealing with the trials of oat varieties it is necessary to say something about the setting up of experimental infective plots as for some few years great difficulty was experienced in their establishment.

An attempt was made in the autumn of 1928 to introduce the oat strain of the parasite into the soil of a field plot by incorporating into shallow drills soil from a field in Aberdeenshire which had carried a "tulip-root" crop of oats during the previous season. Oats grown on this area during 1929 showed one or two typically affected seedlings, but during

the three following seasons, though the disease spread a short distance from the infected end, the infestation shown by oat seedlings was quite light and it could not be considered that the disease had been well established. In the autumn of both 1933 and 1934, oat plants suffering from "tulip-root," which had been received during the preceding spring and summer of each year and had been slowly air-dried, were cut up and put into drills drawn 4 to 5 inches deep in the soil and were afterwards covered with soil. By this means the soil of an area approximately 40 feet long by 21 feet wide was infected with the parasite and a large number of the oat plants grown in 1934 and 1935 showed typical "tulip-root" symptoms. In fact, after the incorporation of the second lot of infective material in the autumn of 1934 it was considered that conditions conducive to the disease had been well established.

In addition to setting up the disease on the field plot it was also successfully established on a small plot within a wire enclosure erected so as to exclude birds which had often played havoc with sowings on the field plot. In the autumn of 1932 and again in 1933 chopped, air-dried, diseased oats were put into shallow drills and then covered with soil. The incidence of disease on oats grown on the plot during 1933 and subsequently has shown that a highly infective medium has been provided. Having in the foregoing manner set up conditions suitable for the study of the disease it was decided to test a series of oat varieties for their susceptibility to attack.

1935 Trials.

These were carried out on the field plot. In October, 1934, three rows of each of the following oat varieties were sown:—*Grey Winter*, *Marvellous*, *Clemrothery* (*Sandy Type*), *Clemrothery* (*Potato Type*), *Pure Line Potato Oat*, *S. 81* and *S. 82*. By the end of March, 1935, inspection showed that there was a fair stand of seedlings of all the varieties and a close examination of the plants was carried out to determine the incidence of "tulip-root" symptoms. During the early part of April, affected plants were brought to the laboratory, carefully torn apart and then dissected as completely as possible under a binocular microscope with a view to obtaining an estimate of the numbers of the parasite present in each. The following notes are based on these observations.

Grey Winter.—All the plants of this variety had a healthy appearance and on close inspection no symptoms of "tulip-root" could be detected.

Dissection of 10 seedlings was carried out and yielded the following numbers of the parasite per plant, 0, 0, 11, 3, 2, 1, 2, 0, 6, 8.

Marvellous.—In the rows of this variety diseased seedlings were very numerous and showed typical symptoms of "tulip-root." Five seedlings were dissected and in each large numbers of the parasite were found as follows, 153, 148, 57, 104, 177.

Clemrothery (*Sandy Type*).—The rows of this variety looked rather better than those of *Marvellous*, but good numbers of typically affected seedlings were found. Five dissected plants yielded the following numbers of the parasite, 185, 50, 54, 51, 194.

Clemrothery (*Potato Type*).—There was a fairly good stand of seedlings but many were found showing "tulip-root" symptoms. Only one plant was dissected, but this yielded 488 examples of the parasite.

Pure Line Potato Oat.—Many plants of this variety were typically diseased and stunted. One dissected seedling yielded 150 worms.

S. 82.—The rows of this variety were irregular and patchy and many typically affected plants were present. One plant showing rather slight symptoms on dissection yielded 120 worms. A second plant showing more pronounced symptoms was found to contain 350–400 worms.

S. 81.—The plants of this variety all had a clean healthy appearance and a close inspection failed to reveal any which could be considered as showing symptoms of "tulip-root." Ten seedlings were dissected and yielded the following numbers of the parasite, 2, 0, 8, 10, 16, 1, 3, 17, 0, 2.

Inspection of the rows of all varieties made later in the season confirmed earlier impressions. *Grey Winter* and *S. 81* remained free from any signs of attack and grew on regularly with the production of good panicles. The other varieties remained patchy with numerous stunted plants many of which, however, particularly of the two *Clemrothery* varieties and the *Pure Line Potato Oat* produced good panicles.

The final impression gained from this season's observations was that *Grey Winter* and *S. 81* were resistant to attack whilst all the other varieties were susceptible.

1936 Trials.

These were conducted on the small plot in the wire enclosure. In April two drills, running the length of the plot, were drawn and short rows, about 1 foot long, were sown of a number of different oat varieties. These included those which had been tested the previous season and

several others the names being as follows :—*Marvellous, New Abundance, Yielder, S. 81, S. 82, S. 84, Grey Winter, Hede, Spet, Summer, Victory, Clemrothery (Sandy Type), Clemrothery (Potato Type), Black Tartarian, Star, Record, Golden Rain, Golden Rain II, Pure Line Potato Oat* and *Eagle. Hede, Spet* and *Summer* are three Swedish varieties which are comparatively resistant to frit fly attack and were included in the trials because of this fact.

By May 8th all the varieties showed a fairly good stand of seedlings and an examination of the plants was begun for symptoms of disease and the presence of the parasite. It had been noticed in the preceding season, when *Marvellous* oats only had been grown on this plot, that an early indication of attack was the presence of small whitish streaks or spickles generally towards the base of one or more of the leaf-blades. They were similar in appearance to those on barley seedlings, parasitized by the narcissus strain of the parasite, described by Hastings, Bosher and Newton (1933).

On teasing up plants showing such spickles the parasites were occasionally found in the tissues of the leaf-blade itself in the vicinity of the spickle, but more frequently in the tissues of the lower, ensheathing portion of the leaf below the ligule. The first examination and dissection of seedlings was therefore carried out when such spickles were apparent. Plants were brought into the laboratory, carefully teased up under the binocular microscope and an estimate made of the numbers of the parasite present in each. A second examination and dissection of seedlings was made about a month later, by which time susceptible varieties were showing typical symptoms of disease. In the case of each variety a single seedling was dissected, note being taken of the presence or absence of symptoms, the presence of discoloured lesions at the base of the plant where the leaves spring from the stem axis and the numbers of the parasites liberated from the tissues. It is to be understood that where a large number of worms was set free from the tissues, the figure for these is of necessity but a rough estimate. One could never be quite certain that all the worms had been liberated as the process of teasing up even a comparatively small seedling is slow and tedious and in order to be sure that all the worms had been dislodged one would have had to be certain that every cell, particularly those abutting on the leaf veins, had been separated from its neighbours. A method whereby the disadvantages entailed in

estimating the numbers of the parasite by dissection of plants was overcome is given later in this paper. In the following notes the dates given after each variety represent the first and second examinations.

Marvellous. (6.V.'36).—A seedling showing a white spickle on the central leaf but not yet showing typical symptoms of disease was found to contain between 20 and 30 adult *A. dipsaci.*

(8.VI.'36). Most of the plants now exhibited characteristic symptoms of "tulip-root." A seedling dissected had short, twisted tillers at the base and the lower part of the plant was swollen and thickened. On dissection, between 250 to 300 adult *A. dipsaci* were found. Very susceptible.

New Abundance. (12.V.'36).—A seedling with a white spickle on the blade of the central leaf was found on dissection to have yellowish lesions at the base of the leaf sheaths and these were irregularly swollen and thickened. About 30 adult *A. dipsaci* present.

(8.VI.'36). Some of the plants in this row now showed typical symptoms with swollen bases and short tillers. On dissecting a seedling, which had split, swollen leaf-bases and three short tillers, about 150 to 200 adult *A. dipsaci* were found. Susceptible.

Yielder. (12.V.'36).—A seedling having a rather swollen base was dissected and found to contain 20–25 adult *A. dipsaci* and many eggs.

(8.VI.'36). Many plants in the row by this date showed marked symptoms. A seedling having a split, swollen base and three or four short twisted tillers on dissection gave more than 400 adult *A. dipsaci* as well as large numbers of eggs. Very susceptible.

S. 81. (12.V.'36).—One seedling was found having a discoloured streak at the base of the lowest leaf. On dissecting this, two adult *A. dipsaci* were found in the leaf-blade. In the lower part of the plant, which appeared to be quite normal, two more adult *A. dipsaci* were obtained.

(8.VI.'36). None of the plants in this row showed any symptoms of "tulip-root" but two had a curious buckling of the lower part of the stem. One of these was dissected but no discoloured lesions were present in the tissues and no specimens of *A. dipsaci* were found. Resistant.

S. 82. (12.V.'36).—All the plants in this row appeared to be distinctly shorter than those of most of the other varieties. One seedling, which had a swollen base and a single twisted tiller, on dissection yielded 20 to 25 adult *A. dipsaci* plus numerous eggs and larvae.

(8.VI.'36). Most of the plants showed marked symptoms of disease. One dissected was very stunted with swollen leaf-bases which were distinctly discoloured. About 200 adult *A. dipsaci* were set free but general decay of the tissues had set in and secondary saprophagous nematodes were also present. Very susceptible.

Grey Winter. (12.V.'36).—A seedling was found with a whitish spickle towards the base of the lowest leaf-blade. This was dissected and in the vicinity of the spickle 2 adult *A. dipsaci* were found but no further specimens were found in the basal tissues of the plant which appeared quite normal.

(8.VI.'36). All the plants except one, which had a buckling of the stem protruding through a split sheath, were free from disease symptoms. One plant having two short tillers was dissected but no examples of *A. dipsaci* were found. Resistant.

S. 84. (12.V.'36).—One seedling showing a whitish spickle on the lowest leaf-blade and some swelling of the stem base on dissection yielded 16 adult *A. dipsaci.*

(8.VI.'36). Many plants in the row showed typical "tulip-root" symptoms. A seedling with a much swollen base and four short tillers contained more than 200 adult *A. dipsaci* and many eggs. Very susceptible.

Resistance. (13.V.'36).—A seedling showing good growth but with a rather swollen base on dissection had a few small yellowish lesions in the basal region and yielded 20 adult *A. dipsaci.*

(8.VI.'36). Many plants showed typical symptoms, though not perhaps of so pronounced a character as some other varieties. A plant with a thickened base and stunted tillers was found on dissection to have brown discoloured lesions and yielded 150 to 180 adult *A. dipsaci.* Susceptible.

Hede. (13.V.'36).—A seedling having a whitish spickle on the blade of the lowest leaf, but showing no other symptoms of attack, on dissection yielded 13 adult *A. dipsaci.*

(9.VI.'36). Many plants in the row now had well marked symptoms. One stunted seedling having 5 short tillers, 2 of which were twisted, was found on dissection to have the basal tissues much discoloured and inflated. It contained more than 300 adult *A. dipsaci.* Very susceptible.

Spet. (13.V.'36).—A seedling having a long white spickle on the lowest leaf-blade and the base of the plant rather swollen was found on dissection to contain 17 adult *A. dipsaci.*

(9.VI.'36). Many plants showed typical symptoms. One seedling very swollen at the base and possessing 4 weak tillers, two of which were twisted, on dissection had brown discolouration at the insertion of the leaf-bases. It contained 250 to 300 adult *A. dipsaci.* Very susceptible.

Summer. (14.V.'36).—A seedling having a white spickle on the lowest leaf-blade, a somewhat rough and swollen base with a buckled tiller, on dissection yielded 10 adult *A. dipsaci.*

(9.VI.'36). Many plants showed typical symptoms. One seedling with a swollen base and a bunch of twisted tillers, on dissection revealed brown discolouration of the tissues at the attachments of the leaf-bases. In these lesions 150 to 200 adult *A. dipsaci* were found. Susceptible.

Victory. (14.V.'36).—A seedling with two or three white spickles on the lowest leaf but of good general appearance and but little swollen at the base, on dissection yielded 25 adult *A. dipsaci.*

(9.VI.'36). Many plants showed characteristic symptoms. One dissected had a swollen base, split leaf sheaths and several stunted tillers. Brown discoloured lesions were present and in these more than 200 adult *A. dipsaci* were found. Susceptible.

Clemrothery (*Potato Type*). (14.V.'36).—A seedling of fairly straight growth and but little swollen at the base, on dissection yielded 17 adult *A. dipsaci.*

(9.VI.'36). Most of the plants exhibited typical symptoms. A seedling having a swollen base and 4 short tillers was found to have brown discoloured lesions at the leaf-bases and contained from 150 to 200 adult *A. dipsaci.* Susceptible.

Clemrothery (*Sandy Type*). (15.V.'36).—The seedlings at this time did not show much sign of attack. One plant without leaf spickles but with a rather swollen base yielded 12 adult *A. dipsaci* on dissection.

(12.VI.'36). Most of the plants by this time showed typical symptoms with much swelling of the base and short weak tillers. One plant dissected, which had two weak tillers, had the usual brown discolouration of the leaf-bases and yielded 350 to 400 adult *A. dipsaci.* Very susceptible.

Black Tartarian. (14.V.'36).—A seedling with a white spickle on the blade of the lowest leaf had 3 adult parasites in the vicinity of the lesion. The base of the stem was swollen and rough and on dissection 30 adult *A. dipsaci* were found.

(10.VI.'36). Most of the plants showed pronounced symptoms of disease. A plant dissected had a swollen base and 4 weak tillers. Internally there were brown lesions at the base of the stem with much inflation of the lower ends of the leaf sheaths. About 400 adult *A. dipsaci* were liberated as well as some hundreds of eggs. Very susceptible.

Star. (14.V.'36).—A straight seedling with white spickles on the lowest leaf-blade and slightly swollen base yielded 9 adult *A. dipsaci.*

(10.VI.'36). Many plants showed typical symptoms. One plant dissected had a swollen base with 5 tillers one of which was twisted. There was not much discolouration of the tissues nor much inflation of the leaf sheaths. 80 to 100 adult *A. dipsaci* liberated. Susceptible.

Record. (14.V.'36).—One seedling dissected had a few white spickles on the lowest leaf-blade and the base of the plant was a little swollen and rough. It yielded 20 adult *A. dipsaci.*

(10.VI.'36). Some of the plants showed typical but not very marked symptoms. One plant examined had a main stem about 18 inches high with 3 basal tillers, all of which were swollen. There was not much brown discolouration of the tissues but 150 to 200 adult *A. dipsaci* were found. Susceptible.

Golden Rain. (15.V.'36).—A seedling showing white spickles on the lowest leaf-blade had a swollen base. The outermost leaf sheath was split and through the slit an inner leaf-base protruded. On dissection 28 adult *A. dipsaci* were found.

(10.VI.'36). Many of the plants showed typical symptoms but others were but little affected. One plant dissected was swollen at the base and had 4 short tillers. Internally brown lesions were present at the leaf-bases and there was inflation of the tissues. 100 to 150 adult *A. dipsaci* present. Susceptible.

Golden Rain II. (15.V.'36).—A short seedling with white spickles on the lowest leaf-blade and the base of the plant much swollen and split with inflation of the leaf sheaths, yielded 75 adult *A. dipsaci.*

(12.VI.'36). Practically all the plants showed marked symptoms of disease and many were much stunted. One plant on dissection was found to have brown lesions and much inflation of the leaf-bases. It yielded about 400 adult *A. dipsaci*. Very susceptible.

Pure Line Potato Oat. (15.V.'36).—A seedling having white spickles on the lowest leaf-blade, the stem base swollen and split yielded 40 adult *A. dipsaci*.

(12.VI.'36). Most of the plants showed typical symptoms. One seedling dissected was much swollen at the base where the tissues showed brown discoloured lesions. More than 200 adult *A. dipsaci* present. Susceptible.

Eagle. (15.V.'36).—A seedling dissected was short and stunted with a swollen base and a few twisted tillers. It yielded 70 adult *A. dipsaci*.

(12.VI.'36). Many plants showed typical symptoms. One dissected had a very swollen base with split sheaths and 4 tillers, 2 of which were weak. There was considerable discolouration and inflation of the tissues and about 400 adult *A. dipsaci* were found. Very susceptible.

Consideration of the foregoing findings shows that the only varieties resistant to attack and manifesting no symptoms of disease are *Grey Winter* and *S. 81*, i.e., the same two varieties which proved resistant in the 1935 trials. Dissected plants of these two varieties contained very few examples of the parasite whereas all the other varieties tested, including those which had proved susceptible in the 1935 trials showed more or less severe symptoms of disease and contained large numbers of the parasite.

1937 Trials.

The results presented are from sowings on the plot in the wire enclosure. Early in October, 1936, a number of winter oat varieties were sown in short lengths in a drill drawn in the plot as follows : *Grey Winter, Black Winter, S. 81, S. 82, S. 147, S. 172, S. 173, 109/1/4* and *ex.* (*109/1*)*1/1*.

Towards the end of March, 1937, a second drill was drawn in which sowings were made of the following varieties of spring oats : *24/2, 30/2, S. 174, S. 175, Bountiful, Binder, King, Great Mogul, Onward, Blainslie, Beseler's Prolific* (*I*) and *Beseler's Prolific* (*II*).

It is not proposed to give the results obtained from all these varieties in the present paper, but to restrict the findings to those from *Grey Winter, S. 81* and *S. 82*, since the first two again proved resistant to attack whilst *S. 82* again suffered severely from disease. It is hoped to present

the results from the other winter and spring varieties when these have been tested further. In the meantime, it may be noted that some of them show distinct indications of resistance to attack.

During the present season's work a technique was employed in the examination of seedling plants whereby many of the weaknesses involved in the dissection of fresh plants and the estimation of numbers of eelworms liberated have been overcome. By making use of a new staining method, which the writer has devised (*vide* Goodey, 1937), it has been found possible to stain the parasites within the tissues of the host and afterwards to make counts of them in situ. The procedure adopted was as follows. On May 4th, when plants of *S. 82* were showing typical symptoms of disease, five plants each of all the winter varieties were dug up. As far as possible plants showing symptoms of disease and others without marked symptoms were taken. The roots were washed to remove soil, each lot of 5 seedlings was suitably labelled and the whole were then fixed in boiling acetic acid-copper acetate so as to preserve the green colour of the plants. They were then washed in water and afterwards taken up through graded alcohols to 70% alcohol in which they were stored.

Individual seedlings were then carefully torn apart by stripping each leaf down to the base of the stem and the material thus obtained was stained overnight in a saturated solution of Scarlet R. in 70% alcohol, one plant at a time being dealt with. The material was next removed from the stain leaf by leaf and shaken up for a few minutes in a corked tubed containing iso-butyl alcohol in order to extract the bulk of the stain. Each leaf was then examined under the binocular microscope in a Petri dish of iso-butyl alcohol. Specimens of *A. dipsaci* present in the tissues were stained bright red and a count of their numbers was made. By this method a more accurate estimate of the numbers of the parasite in each seedling was obtained than by the liberation of living worms from fresh tissues by teasing with needles. Another advantage of the employment of this technique is that it has enabled one to locate even isolated single, individual parasites which by the teasing method would easily have been overlooked. A further point is that the numbers of the parasite found give an indication of the degree of infestation at a given date since the seedlings are all of the same age, whereas in the examination of fresh material the labour and time involved is considerable and must of necessity be spread over several days.

NUMBERS OF *A. dipsaci* IN SINGLE SEEDLINGS.

Seedling	*Grey Winter*	*S. 81*	*S. 82*
1	2	1	519
2	0	3	256
3	0	4	430
4	1	54	165
5	2	4	206

In the foregoing table setting out the numbers of the parasite found in five individual seedlings it is at once obvious that plants of *Grey Winter* contained very low numbers. Two of the five plants had no examples, one had one and two had two each. In the case of *S. 81*, four out of the five seedlings also contained low numbers, but one of them contained 54 eelworms. It may be noted, however, that this particular seedling showed no symptoms of disease and the 54 worms were present in two of the outermost leaves and their ensheathing bases. They had not established themselves in the base of the stem and because of this the plant was free from symptoms. In all 5 seedlings of *S. 82*, disease symptoms were obvious and each plant contained large numbers of the parasite. The third season's observations confirm those of the two previous ones and point clearly to the fact that *Grey Winter* and *S. 81* are to all intents and purposes resistant to " tulip-root " caused by the oat strain of *A. dipsaci* whereas *S. 82* is very susceptible to it. From the fact that some examples of the parasite were found in seedlings of *Grey Winter* and *S. 81*, it may be inferred that the plant does not present an insuperable barrier to the entry of the parasite. It would seem rather that the host tissues do not afford a congenial medium for its growth and multiplication and in this sense the plant is resistant to attack. In the case of susceptible varieties, on the other hand, the parasite having entered the host, finds the tissues favourable to growth and multiplication with the result that these are more or less heavily invaded and characteristic symptoms of disease are set up.

In addition to *S. 82*, the following oat varieties have been found to be susceptible to " tulip-root " :—*Marvellous, New Abundance, Yielder, S. 84, Resistance, Hede, Spet, Summer, Victory, Clemrothery* (*Sandy Type*),

Clemrothery (Potato Type), Black Tartarian, Star, Record, Golden Rain, Golden Rain II, Pure Line Potato Oat and *Eagle.*

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Explanation of Plate.

Photograph of three varieties of oats taken when the panicles were open. *S. 81* (S S 1 on plate) and *Grey Winter* (G W) show clean straight stems without basal thickening and numerous panicles. *S. 82* shows marked stunting with much basal swelling of the stems and no panicles were produced.

GOODEY.—Susceptibility of oat varieties to "tulip-root."

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On some New Hosts of the Stem Eelworm, *Anguillulina dipsaci.*

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Introduction.

During the past two or three years the writer has had an opportunity of making observations on certain plants which are new hosts of the stem eelworm, *Anguillulina dipsaci.* In the present paper four such plants are dealt with, three of which are cultivated garden plants and one a common weed. Of the garden plants, one is a useful food plant, namely the parsnip (*Pastinaca sativa* L.) and the other two are Primulas, namely *Primula pulverulenta* Duthie and *P. polyantha* Mill. (polyanthus primrose). The weed is swine-cress or wart-cress (*Coronopus Ruellii* All., syn. *Senebeira Coronopus* Poir.)

Hosts.

1. Pastinaca sativa L. (parsnip). *Occurrence.*—Diseased plants from a crop growing close to Biggleswade, Bedfordshire, were sent to the writer in July 1935 by Mr. J. W. Dallas, Agricultural Organiser for the County. Mention of this same infestation was made by Petherbridge (1935) but as his note occurs in an official publication of the Ministry of Agriculture having only a limited circulation, it seems desirable to set down some further details of the outbreak of disease on this crop. From information supplied by Mr. Dallas, it appears that the parsnips in question were grown on land which the previous season had been cropped with onions and that these also had suffered from disease due to *A. dipsaci.* This transference of the parasite from onion (Liliaceae) to parsnip (Umbelliferae) is of considerable interest as it is in line with observations made in the United States of America where Godfrey & Scott (1935) reported a similar transference of *A. dipsaci* from garlic (Liliaceae) to parsley (Umbelliferae). On the practical side also it shows the danger of growing parsnips (or parsley and salsify reported by Godfrey & Scott) on land infested with the onion strain of the parasite. At the same time it reveals

the need for further information as to what crops can be grown with safety on such land.

Symptoms.—The affected plants had poor thin roots and showed signs of disease at the crown of the root and in the foliage. The top inch or so of the crown, which is really of stem origin, was in most cases coloured a dirty light brown instead of being cream and the sides were much split and furrowed with considerable necrosis of the tissues. The parasite was abundant in these areas. Some of the leaf-stalks were much stunted, their bases were considerably swollen and some showed longitudinal splits. A noticeable feature of the attack was the pronounced crinkling of the leaf-blades attached to these short swollen leaf-stalks in which the parasites were found in large numbers.

Some of the diseased plants at the time of their arrival were treated with copper acetate-acetic acid in order to preserve the green colour of the foliage and were subsequently stored in 5% formalin. Portions of affected leaf-stalk and crinkled leaf-blade have recently been submitted to the writer's new method (Goodey, 1937) for staining nematodes within plant tissues by means of Scarlet R. Mounts of this material show that the main seat of attack in the leaf-stalks is in the ground tissues just below the epidermis where there is considerable hypertrophy of the cells. In the leaf-blades it is noticeable how the parasites travel up the underside of the midrib and the main branch veins, again just under the epidermis, in which they give rise to much inflation. In some places large numbers of worms can be seen lying side by side just under and at the sides of a vein whilst they are more sparsely scattered in the tissues of the leaf-blade between the branch veins. It is clear from this stained material that the main line of invasion into the leaf is via the soft tissues under the veins ; not, of course, within the vascular elements themselves. Another point also seems clear namely, that as the parasites increase in numbers, it is the larval forms which wander from the region of the veins into the mesophyll tissues, again on the underside of the leaf. The crinkling of the tissues is clearly a reaction phenomenon due to the presence of the parasites or the substances poured out by them.

2. PRIMULA PULVERULENTA Duthie. *Occurrence.*—The plant examined was one of a small number of diseased seedlings which had been growing in a frame in a garden at Southampton. These plants were sent by the raiser in May, 1936, to the Ministry of Agriculture Plant Pathological

Laboratory, Harpenden, for a determination of the cause of disease and the writer received one of them for confirmation of the presence of *Anguillulina dipsaci*.

Symptoms.—The chief sign of disease was the stunted character of the growth. Instead of the outer leaves of the crown being of normal length they were only 1 to 1½ inches long. The bases of the leaf-stalks were much swollen and there was a noticeable inflation of the soft tissues on the underside of the stalk, along the midrib and the principal branch veins of the leaf. This inflation was not confined to the lower side of the leaf but was found on the upper side as well over the veins. Most of the material was fixed in hot 80% acetone and subsequently processed according to Godfrey's (1935) method with Flemming's solution. The results obtained were very striking as when one of the leaves, complete with swollen leaf-stalk, was finally cleared and mounted in Canada balsam, it showed a heavy invasion by the parasite essentially similar to that already described for the parsnip. Large numbers of adult nematodes are present in the soft inflated tissues around the main veins of the stalk and the leaf-blade. Whereas, however, in the parsnip leaf they are chiefly found on the underside of the veins, in the *P. pulverulenta* leaf they occur above as well as below the vascular bundles. Where invasion of the mesophyll between the branch veins has taken place the parasites are chiefly larval forms. It is also noticeable that large numbers of eggs have been laid in the soft tissues of the stalk. As is so often the case, no exact information is available as to the strain of the parasite attacking these seedlings but there can be no doubt of the fact that the parasites had firmly established themselves within the tissues and were causing serious harm to the host.

3. PRIMULA POLYANTHA Mill. (polyanthus primrose). *Occurrence.*—The diseased material of this host also consisted of seedlings which had not yet reached the flowering stage. They had been raised from seed during the spring and summer and had been planted out in two narrow borders in a private garden at Kendal, Cumberland, in the autumn of 1936. Affected plants were sent to the Ministry of Agriculture Plant Pathological Laboratory, Harpenden, in the first instance, about the middle of February 1937. Shortly afterwards a further supply of fresh material was sent to the writer. No information is available as to what previous occupants of the borders might have furnished the parasites invading

these polyanthuses. In view, however, of the observations which are presented later on the presence of the parasites within the rudimentary flower buds, the possibility is not excluded of the infection being seed-borne.

Symptoms.—The roots of the diseased seedlings appeared to be perfectly normal and healthy but the crowns were composed of dwarfed leaves many of which showed marked inrolling of the leaf edges on their backs. There was no pronounced swelling or inflation of the superficial tissues at the base of the leaf-stalks. Many of the small leaves exhibited considerable inflation of the epidermis on the underside but whether this was an abnormal condition due to the parasite cannot be safely assumed. On examining some of the dwarfed leaves in water under a dissecting microscope, a number of small, reddish brown lesions were found along the midrib and at many scattered spots on the leaf-blades. From such lesions, or their immediate vicinity, numbers of *A. dipsaci* were teased out with needles. In one small lesion, scarcely visible to the naked eye, a compact nest of 18 adult worms was found. Several adult worms were also found on teasing up the sepals of a small rudimentary flower bud from the centre of a crown.

Some of the material was treated with copper acetate-acetic acid to preserve the green colour and was afterwards stained with Scarlet R. in 70% alcohol. Examination of this material showed that the parasites had not effected a general invasion of the leaves via the tissues of the leaf-stalk such as was found in the case of parsnip and *P. pulverulenta*. Only small, isolated groups of eelworms were found in the leaf-stalks. There was also no general spread of the worms from the region of the veins into the intervening tissues of the leaf-blades. It would seem from the scattered character of the small lesions found on the underside of the leaves, that the parasites had invaded the host tissues at many isolated spots. Examination of many flower buds from the crowns of plants stained with Scarlet R. showed that there was a plentiful invasion of the tissues of the inflorescence. The parasites were present in good numbers in bracts subtending individual flowers, in the receptacle, sepals, petals, and anthers. It is clear that if such infected buds succeeded in growing and the flowers bloomed, the whole structure would be carrying the parasite probably within the tissues of the ovary or at any rate in the receptacle. Even if the developing seed was not itself actually parasitized, there

would be every likelihood that, as the seed ripened and the seed capsules dried, the parasite would reach its resistant, infective stage and so could easily be distributed with the seed when this was harvested.

4. Coronopus Ruellii All. (swine-cress or wart-cress). *Occurrence.*—Infected plants were found growing at Winches Farm, St. Albans, on a small experimental plot devoted to the cultivation of daffodils parasitized by *A. dipsaci*. It had been noticed that the leaves of many of the affected narcissi had shown typical " spickles " due to the parasite during the spring of 1936. The narcissus foliage withered and died down on the surface of the soil during May and June and weeds were allowed to grow. Early in July it was noticed that some plants of swine-cress, which is a common prostrate weed on this and adjacent plots, exhibited a few irregular twisted leaflets. On teasing up some of these in water, adults of *A. dipsaci* were found in the lesions. There can be no doubt, in the writer's opinion, that the source of infection was the narcissus strain of the parasite which had migrated from the withered infective narcissus foliage lying on the surface of the ground.

It is worth putting on record that on this plot also a plant of *Plantago major* L. (greater plantain) was found with yellowish leaves which were lightly infected with the parasite. *P. major* is already known as a host of the narcissus strain of *A. dipsaci* from the observations of Hodson (1929). The present record of attack on swine-cress, a member of the Cruciferae, increases still further the already fairly wide range of alternative hosts of the narcissus race of the parasite.

Symptoms.—The leaves of *Coronopus Ruellii* are pinnately divided once or twice and normally the small wedge-shaped segments are flat and regular. It was the presence of a few irregularly twisted segments on some of the plants which first attracted the writer's attention. Closer examination of these showed the irregularity to be due to the midrib being curved back on itself. Specimens of affected plants were fixed in July 1936 in copper-acetate-acetic acid and subsequently stored in 5% formalin. In February 1937, one or two of the lesions were treated with Scarlet R. in 70% alcohol and on mounting these in Euparal the presence of the parasites was beautifully revealed and further light was thrown on the character of the lesions. The worms occur in the soft tissues under the midrib in good numbers. From the midrib they invade the tissues of the leaflets and here give rise to small scattered lesions, many of which show complete perforation of the

tissues. The stained preparations also reveal the fact that invasion of leaflets may take place at isolated spots far removed from an infected midrib as microscopic lesions have been found close to the tips of leaflets with a single parasite in each.

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On the Eelworm Disease of Primulas caused by *Anguillulina dipsaci*, Kühn.

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In Britain, the year 1937 is likely to be memorable in the history of cultivation of many species and varieties of plants belonging to the genus *Primula*, as witnessing, for the first time, attacks of great severity by the nematode, *Anguillulina dipsaci* in a number of plantations of Primulas, reducing the vitality of the plants wherever it occurred, and in many instances causing complete destruction of certain species. The plants classed as members of the Candelabra Section, which includes some of the finest and most extensively grown species and varieties in the whole genus, proved particularly susceptible but, while members of many of the other Sections of Primulas did not escape attack, the injury inflicted was not usually of such high order of severity.

The first serious outbreak in 1937 was discovered by the writer as the result of a critical examination, towards the middle of May, of some diseased specimens of *Primula japonica*, A. Gray, from Mr. A. Harrison, N.D.H., Horticultural Instructor to the Glamorgan County Council. The plants had been growing at the Educational Gardens, Singleton Park, Swansea, and a microscopical inspection of portions of their leaves and flowering stems disclosed the presence of *Anguillulina dipsaci* in all stages of development from egg to adult. Subsequent visits to this centre and other affected gardens in South Wales and Monmouthshire, established the fact that the trouble was widespread to varying degrees of intensity, and since the occurrence of the parasite on any species of the genus *Primula* or, in fact, on any member of the Natural Order, Primulaceae, has never been the subject of a scientific investigation, it was thought advisable to give an account of certain aspects of the research work carried out by the present writer in this direction.

During the course of this work twenty-three previously unrecorded species as well as seven subspecies of Primula were discovered to be

attacked by *Anguillulina dipsaci* for the first time. In addition, the susceptibility of *Primula rosea, P. vulgaris, P. japonica,* and *P. helodoxa* to this nematode was also confirmed (Goodey, 1933).

Species of Primula Liable to Infestation.

The extensive observations made by the writer on infested plantations of Primulas, in South Wales and Monmouthshire, during the past three years, indicate that the following species are liable to attack by the nematode. The arrangement adopted in the classification of the different species into distinct Sections is based on the scheme approved by the last Primula Conference held in 1928 under the auspices of the Royal Horticultural Society (1929). Each subspecies is denoted by an asterisk.

SECTION—CORTUSOIDES, Balf. f.

P. cortusoides, Linn ; *P. saxatilis*, Komarov ; *P. lichiangensis*, G. Forrest* ; *P. Veitchi*, Duthie* ; *P. seclusa*, Balf. f. et Forrest* ; *P. latisecta*, W. W. Sm.

SECTION—MEGASEAEFOLIA, Balf. f.

P. megaseaefolia, Boiss.

SECTION—VERNALES, Pax.

P. Juliae, Kusnetzow ; *P. vulgaris*, Huds.

SECTION—PETIOLARES, Pax.

P. Winteri, W. Wats.

SECTION—FARINOSAE, Pax.

P. farinosa, Linn ; *P. rosea*, Royle ; *P. frondosa*, Janka ; *P. luteola*, Rupr.

SECTION—DENTICULATA, Balf. f.

P. denticulata, Sm.

SECTION—CAPITATAE, Pax.

P. Mooreana, Balf. f. et W. W. Sm.* ; *P. crispata*, Balf. f. et W. W. Sm.*

SECTION—NIVALES, Pax.

P. chionantha, Balf. f. et Forrest.

SECTION—SIKKIMENSIS, Balf. f.

P. sikkimensis, Hook ; *P. pseudosikkimensis*, G. Forrest* ; *P. microdonta*, Franch ex Petitm ; *P. Florindae*, Ward ; *P. vittata*, Bur. et Franch.

SECTION—CANDELABRA, Balf. f.

P. Beesiana, G. Forrest ; *P. burmanica*, Balf. f. et Ward ; *P. aurantica*, W. W. Sm. et Forrest, and its hybrids ; *P. Bulleyana*, G. Forrest ; *P. pulverulenta*, Duthie, and its varieties ; *P. japonica*, A. Gray, and its varieties ; *P. helodoxa*, Balf. f. ; *P. anisodera*, Balf. f. et Forrest ; *P. Wilsoni*, Dunn* ; *P. chungensis*, Balf, f. et Ward.

SECTION—AURICULA, Duby.

P. viscosa, All.

Circumstances Attending Outbreaks of the Parasite.

It is probable that many plantations of Primulas are never quite free from the parasite, but it is only under exceptional weather conditions that it assumes the form of an epidemic, or does any considerable amount of damage. Exceptional climatic conditions prevailed in the first half of 1937. The rainfall during the winter and earlier part of Spring had been well above the average, followed by very low soil temperature and dry atmosphere in May and June. These circumstances seriously crippled the plants and later checked their normal active growth at the time of the year when multiplication of the parasite is about its height. In this connection the following account in a letter received from a Glamorgan grower in July 1937 is of considerable interest. " . . . . . In some years in my beds of Candelabra Primulas, I lose a small percentage of plants which die off during the early summer months. This season we have had, however, a most disastrous time—they were a wonderful looking crop until they started dying off some six weeks ago. I should say we have already lost about 70 per cent. of the plants. I enclose under separate cover a few typical diseased specimens for your opinion and report . . . " The specimens sent were heavily attacked by *A. dipsaci* and exhibited the characteristically distorted and deformed appearance.

While by no means confined entirely to plantations of Primulas on ground which is of a sandy character, outbreaks of attack do appear to be favoured by dry soil conditions. The general experience gained in the course of extensive observations made in 1937 was that if there is a dry patch in a plantation, the plants are likely to be more severely attacked there than in any other part. Further, that side of a plantation which gets most sun will frequently show a greater number of diseased plants.

At one of the centres under observation in 1937, a proportion of the stock of each species of the Primulas belonging to the Candelabra Section had been planted in damp ground along the banks of a stream, being well shaded from the intense heat of the sun by large trees. The remainder in each case had been planted in a wide border, specially prepared for the purpose, on a considerably drier and more exposed situation. All the Primulas, amounting to several hundreds, planted in this latter position died off between the middle of May and the end of July, and although eelworms were also present in the tissues of the plants alongside the stream, they were not in sufficient numbers to cause the general symptoms or to materially alter the growth. In fact, the only indication by which the attack could be detected was that the lower leaves of some plants showed signs of chlorosis, while the later growths were somewhat stunted.

Sources of Infection.

Consideration of the general observations made on infested plantations indicates four main sources of infection of potential host-plants.

The first comprises of the dispersal of the nematode to fresh sites by the active wandering of the pre-adult larvae in the soil, and by the transport of contaminated soil adhering to the roots of plants, garden tools and the boots of workmen.

The second source of infection consists of infective larvae which remain quiescent in desiccated remains of attacked plants. It is a common practice among some growers to remove dead leaves and sickly-looking plants periodically and to burn them. At the time of the writer's visit to one centre, this was being done, the fire being at one side of the plantation. However, a few diseased plants had been lying on the ground and on the rubbish heap for about three weeks and upon examination of some of the leaves large numbers of the nematodes were found to be present. It is evident, therefore, that if diseased portions of the plants are not destroyed immediately, but are left lying about in the gardens, they serve for the continued development and dissemination of the parasite. It has also been found that the nematode can persist and retain its vitality in the foliage of Primulas even when the leaves in which it is living become desiccated. Some leaves in a shrivelled-up, brown condition were brought into the laboratory in May and allowed to become thoroughly air-dried at room temperature. In the following November,

portions of the leaves which had been crushed into fine powder were placed in water in glass capsules. Microscopic examinations some hours later revealed that in each case numerous larvae had been liberated into the water. These larvae displayed active motility and had thus remained reviviscent for a period of six months.

The third source of infection and probably the most frequent method by which the eelworm is spread into fresh districts is by the introduction of infected plants from nurseries and gardens. Many affected plants are unwittingly introduced into clean areas, the reason being that the true character of the disease has not hitherto been known to the Primula grower and that plants in instances of light infestations do not show any marked pathological conditions. It is highly probable that fresh infections brought about in this way, though at first may involve but a few isolated plants in a plantation, would spread over large areas in a comparatively short time, as in the case of the Potato and Narcissus strains of this eelworm. That the distribution of infected plants is a common mode of dissemination of the parasite on Primulas is well exemplified in a letter received from a Monmouthshire grower in early May 1937. "I am sending you the enclosed diseased Primulas, part of a stock delivered here this morning from . . . . . You will note that the leaves are going rotten at the base. There are several others like them in the consignment . . . . . " The specimens were found to be attacked by *A. dipsaci*.

The fourth mode of infection, but as yet not definitely proved, is by means of sowing seed saved from infected plants. Although eelworms have not been discovered actually in or upon dry seeds of any species of the genus *Primula*, the writer has recently found that samples of seed from badly affected plants are capable of yielding the parasite in a living condition when soaked in water. Further, it would seem from enquiries made into the history of the several outbreaks investigated in 1937, that new foci of infection may become established by the sowing of seed saved from infested plants. Thus in three of the outbreaks investigated the plants had originally been obtained from seed supplied from a nursery where the stocks of Primulas, upon examination by the writer in 1937, were found badly attacked. Each of the growers concerned with these outbreaks had grown his own seedlings separately and for this reason the occurrences of the disease at the three centres, which were incidentally

located several miles apart, cannot be accounted for on the supposition that they originated from distribution of plants from a single and common stock of affected seedlings. The incidence of the disease is, in fact, rather difficult to explain except on the assumption that the parasite was already present at the three centres or, that the seed, as sown, carried the nematode on it or in it, in a resting condition. It is significant in this connection that the Primulas at one of the centres involved were in their first year of growth and growing on newly cultivated land, specially prepared for the purpose and which was previously permanent pasture. That seed dissemination is the normal mode of spread adopted by *A. dipsaci* when occurring on certain other host plants, in particular Compositae, is well known, consequently in view of this fact and a consideration of the foregoing observations, the adoption of the precautionary measure of exercising great care in the selection of perfectly healthy stocks of Primulas for seed production might well be considered.

Symptoms of Attack on Primulas.

Any conditions that affect Primulas adversely tend to induce symptoms that may readily be mistaken by the average grower for those resulting from attack by *A. dipsaci*. Thus, most species of Primula, being in their native habitats accustomed to surroundings entirely reverse to our wet winters and dry summers, are liable to be damaged by severe drought in late spring or in the early summer months. This may kill the plants outright, whilst less severe conditions may cause a high proportion of the lower leaves to turn yellow and wilt. Drought is especially liable to do harm when following a wet winter. An attack by leatherjackets, *Tipula* sp. may cause the plants to die back and similar effects are seen when the plants are injured by cutworms, *Agrostis* sp. Hence great care is necessary to distinguish between symptoms due to invasion by *A. dipsaci* and those due to adverse environmental factors or injury inflicted to the tissues in the basal regions of the leaves and flowering stems by certain insect pests.

The general behaviour of infected plants suggests the lack of vigour accompanied in late spring and during the summer months by discoloration of the leaves and considerable hypertrophy of the tissues, particularly in the region of attachment of the leaf-stalks and flowering stems to the crown or root system of the plant. In addition, there is

usually much distortion or deformation of the flowering stem so typical of attack by *A. dipsaci* in other host plants (see Plate). If the plant is small when attacked, or is growing under unfavourable conditions, it usually succumbs with the advent of dry weather in the earlier part of the growing season.

I.—*Leaf Symptoms.*

The pathological manifestations produced in the leaves show a wide range of variation, depending on the species of Primula attacked and the severity of the infestation. Further, no single leaf symptom shown by the different species can be regarded as specifically attributable to attack from *A. dipsaci*, marked twisting and thickening of the leaves, so characteristic of infestation by the nematode on narcissus, phlox and other plants being almost non-existent. An exception must be noted, however, in the case of an attack on *P. megaseaefoliae*. In lightly affected plants of this species the midrib and the secondary veins are sharply defined, being brown or black in colour, thus delimiting the mesophyll tissue into distinct areas. The appearance of the leaves at this stage is reminiscent, on the whole, of the blotched condition in the leaves of a fern, *Pteris* sp., when invaded by *Aphelenchoides olesistus*. In the extensively diseased plants of *P. megaseaefoliae*, there is in addition to the darkened venation a diffused discoloration of the parenchymatous tissue bordering the main veins followed later by general browning and shrivelling up of the entire leaf.

In the case of the other species found seriously affected, except *P. anisodera* and *P. Wilsoni*, the sequence of the various stages in the manifestation of the disease was essentially as follows :—

First the affected leaves develop a pale green or yellow discoloration at the edge, which later becomes brown.

This is followed by the appearance of pale green or yellow areas in the leaf accompanied by gradual extension inwards of the discoloration at the edge. The latter may proceed more rapidly at some particular region in the leaf, most frequently the tip, resulting in puckering of the entire leaf due to shrinkage of the tissues at the affected part.

The leaves at this stage on being gently pulled are usually found to break away readily at their attachment with the crown of the plant on account of decay and the collapsing of the tissues at this point.

Finally, the entire leaf turns brown or black, becomes dry and shrivelled up, frequently with incurved or recurved margins, depending on the species of the plant attacked.

In *P. anisodera* and *P. Wilsoni*, the development of the various stages in the manifestation of the disease in their leaves was found to differ from that in all the other species examined :—

At first the leaf-stalk and the midrib become yellow and later brown.

This is followed by the appearance of diffused, brown discoloration of the mesophyll tissues bordering the main vein. The discoloration gradually spreads outwards, particularly along and involving the secondary veins, towards the edge of the leaf. This process seldom develops at a uniform rate in all directions from the midrib. For instance, part of one side of the midrib often dies off before its corresponding part of the other side, resulting in considerable puckering and twisting of the remainder of the leaf.

The entire leaf becomes brown, crisp and dry and generally rolls upward and inward.

II.—*Flowering Stem Symptoms.*

Many of the abnormalities manifested in the flowering structures are, in contrast to those shown by the leaves, decidedly diagnostic of an attack by *A. dipsaci*. The following are the principal symptoms, and whilst some plants exhibit most of them, others exhibit only two or three :—

The stems are dwarfed (Plate, Fig. 2) and in extreme cases entirely inhibited or so short that the inflorescence is only partially enfolded (marked *a* on Plate, Fig. 2).

The epidermis separates from the tissues beneath, giving rise at first to pale or white pustules, which later become brown or black in colour.

The stems are twisted and distorted (Plate, Fig. 1d).

The stems instead of being smooth and even may show deep, longitudinal furrows due to shrinkage of the underlying tissues (Plate, Fig. 2).

The stems may split, sometimes longitudinally, but frequently at right angles to the long axis of the stem.

The stems are very flaccid as opposed to the firm consistency of normal plants.

The stems are greatly thickened. This may involve the whole stem or in part as in the species with the Candelabra type of inflorescence where the thickening is often limited to an internode between two whorls or umbels of flowers.

In species with the Candelabroid inflorescence, there is often much variation in the length of the internodes between the different whorls of flowers on the same plant. Some internodes may be of normal length whilst others extremely short (Plate, Fig. 1c). In extreme instances, two, three or more whorls of flowers may be crowded together and their respective internodes no longer evident (d and e on Plate, Fig. 1).

In plants with the Candelabroid inflorescence, the number of whorls of flowers are usually reduced and even those present may be incomplete, being represented in extreme cases by about two flowers (d and r on Plate, Fig. 1).

The flowers are small, pale in colour and poor in form compared with those of healthy plants.

The plants fail to set seed, the flowers having wilted and died prematurely, or if seed is set, a large percentage of it is sterile (Plate, Fig. 2).

The plants show in advanced and severe stages of attack a progressive darkening accompanied by decay of the tissues from the base upwards.

Parts of Plant Invaded by the Parasite.

The basal portions of the leaf-stalk and flowering stems are the positions primarily attacked. Later all regions of the leaf, flowering stem and flower are in turn invaded. In the leaf, the eelworms are usually most abundant at first in the major veins and the bordering mesophyll tissues, but later spread to all other parts.

Since much significance is attached to the possibility of seed dissemination as methods of spread of the parasite into fresh districts, flowering stems and the flowers in various stages of development were studied. By examination it was discovered that all regions of the flowering stem and flower stalks or pedicels are attacked, particularly the tissues of the latter in the vicinity of their attachment with the individual flowers. It was often noticed that the flower stalk at this point was discoloured

and in all such cases enormous numbers of eelworms were invariably present and the flower itself having wilted or at the best a poor specimen compared with that of a healthy plant. The parasite has also been found to occur in the sepals, petals and in the various components of the gynoecium but not actually within the ovules. While the nematode has not been observed so far within the walls of an ovule of a Primula, examinations of flowers from diseased plants have revealed vast numbers of worms amongst the ovules and often associated with a completely collapsed condition and decay of a high percentage of these organs. Further, it has been discovered that samples of seed saved from affected plants are capable of yielding the parasite in a living condition when soaked in water.

Susceptibility of Different Species of Primula to Attack.

Different species of the genus *Primula* vary to a marked extent in the degree to which they are liable to suffer from attack by *A. dipsaci*, but so far as is known no species can entirely resist an invasion of their tissues by this nematode. The very large collection of Primulas at the Educational Gardens, Singleton Park, Swansea, afforded an ample opportunity to compare the behaviour of a number of species in this respect. The observations were made on plants growing in an extensive border specially prepared for their cultivation and where great losses occurred in 1937 from infestations of the parasite. The degree of infection was of an exceedingly high and uniform order throughout the whole area, judging by the extent of the damage to plants of certain species that had been arranged in rows extending the full length of the border. An attempt was made to group the different species and subspecies into four divisions, according to the amount of damage done by the eelworm :—

1. Very susceptible species.—*P. pulverulenta, P. burmanica, P. japonica, P. aurantica, P. Beesiana, P. helodoxa, P. Bulleyana, P. anisodera, P. Wilsoni.*

2. Susceptible species.—*P. megaseaefolia, P. lichiangensis, P. cortusoides, P. seclusa, P. Veitchi, P. saxatilis, P. rosea, P. pseudosikkimensis, P. Juliae, P. denticulata.*

3. Resistant species.—*P. Florindae, P. lactisecta, P. crispata.*

4. Very resistant species.—*P. viscosa, P. capitata, P. microdonta.*

It will be noted that the very susceptible species, included in the first group, belong to the Natural Section Candelabra which comprises the more widely grown and decorative members of Primula. At the centre under consideration, all the plants of every species in this first group were killed between the middle of May and the end of July. In the second group, all the plants of each species suffered severe defoliation during the summer months but, on the whole, comparatively few were actually destroyed. In the third group, although the parasite occurred in vast numbers, in all stages of development from egg to adult in many plants of the three species included in this division, nevertheless, marked pathological symptoms were not evident, apart from dwarfing and hypertrophy of certain portions of the lower leaves and, occasionally, the distorted appearance of the flowering stem. In the fourth group, there was no sign whatsoever of an attack on any species despite the fact that in each instance an entry had frequently been effected, at least, into the tissues in the vicinity of the basal regions of the leaves and flowering stems. In no case had the parasite become established, judging by the absence of breeding. It was further noticed that its persistence in these host plants was also of a short duration, inspections earlier than the first week in June or later than the second week of August having failed to reveal its presence in any plant examined.

Observations made on another infested plantation in 1937 have also confirmed the view that the eelworm seems to be able to attack freely some particular species, and, at the same time, appears unable or at least unlikely to flourish on other species. It is realized, however, that definite conclusions cannot be drawn as to the relative susceptibilities of different species of Primula to the disease from data collected in one year, but only from those extending over a succession of seasons and under diverse soil conditions.

Control Measures.

In order to reduce to a minimum the risk of introducing the nematode into areas free from infection, it is recommended that all newly acquired stocks of Primulas whether seed, plants or portions of plants in the form of divisions, should be grown in the first instance in an isolated place. They should then be regarded as being in quarantine until such time as it is possible to make certain that they are free from the parasite. Normally there should be no great difficulty in discerning affected plants

either when in full bloom by the characteristic abnormalities, already described, in the floral structures or, but less readily, by the sickly, unhealthy appearance of the leaves, particularly during dry periods in the earlier part of the growing season.

Where, in spite of precautions, a grower finds his plantations of Primulas infested, the only safe course for him to pursue in the present stage of our knowledge of this eelworm is to localize the outbreak. If heavy losses are to be avoided, it is imperative to lift at once all affected plants together with the apparently healthy ones immediately surrounding them. The plants thus removed should be burnt on the infested ground and not thrown on to the rubbish heap as this may aid in the further spread of the parasite. Since the parasite can remain viable in dry shrivelled leaves, these should also be carefully collected and treated likewise. As far as is known, the only practical means whereby the parasite is likely to be eradicated from infested ground is by starvation. The exact length of time that is necessary cannot be definitely stated, but judging from field observations on the nematode when occurring on Narcissus (Hodson, 1932) a period of at least eighteen months should intervene before Primulas can be safely grown again in the ground.

Acknowledgments.

The writer takes this opportunity of acknowledging his indebtedness to Messrs. D. Bliss, V.M.B., and T. L. Gardiner, for having been allowed access to the collections of Primulas at the Educational Gardens, Singleton Park, Swansea.

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Fig. 1.—Typical examples of Primula flower malformations due to attack by *A. dipsaci*.

Fig. 2.—Typical Primula plant (*P. pulverulenta*) seriously attacked by *A. dipsaci*.

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On a new species of *Stephanofilaria* causing lesions in the legs of cattle in the Malay Peninsula.

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The nematode genus *Stephanofilaria* was created to accommodate the species *S. dedoesi* Ihle and Ihle-Landenberg, 1933, which was found in association with a disease in cattle named "Cascado" in the Dutch East Indies. Two further species have since been described, namely, *S. stilesi* Chitwood, 1934, from skin lesions in cattle in the United States and *S. assamensis* Pande, 1936, from "Hump Sore" in cattle in Assam. The disease and its relation to the nematode has been studied in each case by Bubberman and Kranefeld (1933) in the Dutch East Indies, by Dikmans (1934) in the United States and by Pande (1935) in Assam, and the results of their studies leave little doubt as to the authenticity of Stephanofilariasis as an important skin disease in cattle.

The present paper is concerned with a species of *Stephanofilaria* which was obtained from cattle in Kuala Lumpur, F.M.S., having been extracted from lesions similar to those previously described but differing mainly in their location, which in the cattle examined by the writer was in the lower parts of the legs. This appears to be the most common and possibly the only site of the lesion in the present instance, but an extensive survey of the disease would be required in order to verify this beyond doubt. It is known locally as "Filarial Sore" or "Krian Sore" and both on account of its high incidence and detrimental effects it is regarded as a disease of considerable importance. The origin of the name "Filarial Sore," which is apparently of long standing, could not be ascertained, but the opportunity of establishing the helminthic origin of the term or the disease was kindly provided to the writer in May 1937 by the Veterinary Department, Kuala Lumpur, and resulted in the finding of the parasite described herein.

"Filarial Sore."

It is not proposed here to give more than a brief description of the disease for time did not permit of the study of many cases, or of the incidence of the disease in different localities, or of the history and development of the lesion in individual cases. The "sore" occurs on any part of the legs from just above the hoof to the second joint and may vary from a simple lesion about an inch in diameter to an elephantoid growth about 8 inches long by about 5 inches in circumference. The smaller sores are not much elevated from the general level of the skin surface and are usually bleeding, irritable and somewhat denuded of hair. In larger sores there is a corresponding increase in prominence and in one case the diameter of the diseased part of the leg was increased from the normal by about 2 inches. The surface of such sores is dry and crusty, grey in colour, almost hairless, with bleeding cracks and indentations. It is frequently licked by the cow to allay irritation and is a source of attraction to flies such as Stomoxys and various Muscidae. The sore tissue is vascular and bleeds freely when a portion is cut away. Adult worms were obtained by cutting up this tissue into small pieces in a Petri dish with normal saline. The females could be seen projecting from the cut surface and were easily withdrawn by means of a forceps. Males were obtained in the same way and were also found free in the dish in which the material had been chopped up.

Lesions from three different animals were examined in this way and in each case adult worms were found. Sections were also made of this material and adult worms were demonstrated in the epithelium, which appears to be the exclusive habitat of all the known species of *Stephanofilaria*. I am much indebted to Professor G. R. Cameron, University College Hospital Medical School, London, for the following description of sections from the elephantoid type of lesion illustrated in Plate I.

Professor G. R. Cameron's Report on the Histo-Pathology of a Stephanofilariasis Lesion.

"The material supplied by Dr. Buckley consisted of one section of normal cow skin stained with haematoxylin and eosin and four sections from abnormal skin, two stained with haematoxylin and eosin, and two with van Gieson.

In comparison with the normal skin, that from the cow with elephantiasis show marked hypertrophy of the epidermis, all layers being involved. Large peg-top processes pass down into the dermis occasionally for quite long distances, some showing secondary and tertiary branching. Everywhere the basal layer of cells is well defined and there is no suggestion of neoplastic transformation. Mitotic figures are few in number in the growing layer. In a few places apparently detached epithelial masses are present deep in the corium, but in the absence of serial sections it is impossible to say whether this is a real implantation or purely due to folding. The photographs of the actual process suggest the latter explanation. The thickened epithelial processes are usually in the neighbourhood of areas of surface ulceration. On the other hand the longest processes are around the sites of growth of the parasite and away from the ulcers. A striking feature is the presence of large epithelial-lined cystic cavities, filled with structureless material and in some cases pus. A few of these show loss of epithelium and are beginning to be organised by granulation tissue, rich in foreign body giant cells. Large spindle shaped areas in others suggest the presence of cholesterol crystals. These cysts appear to arise in occluded sebaceous ducts, although it is impossible to exclude the possibility also of implantation epidermoids having been formed.

There is a marked leucocytic and round celled infiltration beneath the epidermis. The round cells are mostly of the macrophage type, although lymphocytes and occasional plasma cells, can be seen. Eosinophil leucocytes are relatively few in number. Polymorphonuclear leucocytes predominate in the bases of the ulcers. The parasites are surrounded by a fairly thick capsule of young granulation tissue in which macrophages and fibroblasts are numerous.

The dermal infiltration is uniform and very marked in its upper third, but in the deeper part is streaky with a distribution confined to the larger blood vessels and their branches. A very striking feature throughout the dermis is the large number of newly formed blood vessels and lymphatics each associated with perivascular collections of lymphocytes and small macrophages. In the deep layers this perivascular distribution is very striking and appears to be chiefly around and in the smaller lymphatics.

The outstanding features of this condition thus appear to be :—

1. Hypertrophy of the epidermis with exaggeration of its papillary processes.
2. Formation of occlusion cysts in the superficial dermis, some of which are infected.
3. Diffuse subacute and acute inflammation of the more superficial layers of the dermis.
4. Perivascular infiltration with lymphocytes and macrophages in the deeper layers of the dermis and in the muscle.
5. Prominent lymphatics.

The process appears to be one of a relapsing inflammation of the dermis with stimulation of growth of the epidermis."

Description of *Stephanofilaria kaeli* sp. nov.

In both male and female worms the cuticle is minutely striated transversely throughout the length of the body and in the anterior part of the worm there is a cuticular " frill " surrounding the body at each striation. Except under a high magnification the cuticular frills are very difficult to see. They become more and more indistinct towards the posterior part of the worm and may be lacking from the tail region. They have been described in *S. dedoesi* but not in the other two species. It is probable, however, that they are characteristic of the genus. The dermo-muscular layer is unusually thick and well-developed, and, considered in conjunction with the backwardly-directed cuticular frills, suggests that the worm is well adapted for movement and migration within the skin tissue. Lateral alae, such as are present in *S. stilesi*, were not observed in the present species, but the cuticular striations are interrupted by a distinct external lateral line.

Female. Four intact specimens were obtained and measured 5·75, 6·9, 9·2 and 9·94 mm. respectively. The smallest of these was immature. The width of the larger specimens was about 150μ and the vulva was 62–98μ from the anterior end, but there was no co-relation between this distance and the length of the worm and therefore this cannot be regarded as a reliable diagnostic character in the present species. The anus, rather difficult to see except under a high magnification, is 25–35μ from the tip of the tail and leads to a very indistinct rectum.

The peri-buccal ring has 15–18 spines arranged in an unbroken circle and the ring of cephalic spines posterior to it is interrupted laterally by

Stephanofilaria kaeli sp. nov.

Fig. 1. Anterior end of female, lateral view.

Fig. 2. Tail of female.

Fig. 3. End-on view of female, shewing peri-buccal and cephalic setae, amphids and cuticular frills.

Fig. 4. Tail of male, ventral view.

Fig. 5. Piece of cuticle, shewing transverse striations and cuticular frills.

Fig. 6. Anterior end of female, low magnification.

Fig. 7. Tail of male, lateral view.

Fig. 8. *A*. Embryonated uterine egg (unstained). *B*. Extra-uterine larva in blood smear from " filarial sore." Stained Giemsa. *C*. Sheathed microfilaria in blood smear from " filarial sore " and also found in peripheral blood from neck of infected cow. Stained Giemsa.

(*N.B.* The scale ·05 mm. refers to all the figures except 3, 5 and 6.)

two large amphids and formed two semi-circular groups of 11 and 12 respectively in one specimen and 9 and 10 in another specimen. These spines are difficult to count accurately unless the head is cut off and mounted so that an end-on view of it can be obtained.

The oesophagus joins the intestine a short distance behind the level of the vulva and is encircled by the nerve ring about the level of the vulva.

The vagina extends posteriorly from the vulva as a stout-walled tube about 0·4 mm. long, and is enclosed by a cellular sheath, one cell in thickness. It is curiously modified at its anterior end where it is loosely enclosed in a pear-shaped sac, inside of which it is connected with a thick-walled distal organ. The two uteri run uncoiled, though somewhat twisted about one another to the posterior quarter of the body where the ovarian tubules are situated.

Male.—Male worms were obtained in much larger numbers than females. Of six taken at random, the average length was 3·25 mm., the largest of these being 3·65 mm. In breadth the body varies from 80–100μ. The peri-buccal ring of spines is similar to that in the female but smaller in diameter and the cephalic spines differ from those in the female only in numbers, comprising 16 to 17 spines, arranged in two semi-circles of 8 each or 8 and 9, interrupted laterally by the amphids. The tail is slightly bent ventrally and the anogenital aperture is about 35μ from the tip of the tail. There is a complex system of papillae in the tail region. Two pairs of large papillae are situated about half-way from the anus and the tip of the tail, close together on the mid-ventral line, and posterior to these is a pair of small papillae. Immediately anterior to the anus in the mid-ventral line is a group of three, one pair of small papillae and between them and the anus is a larger single papilla. There are 6 to 7 pairs of *sub-ventral* papillae extending from about the level of the anus anteriorly to the anterior extremity of the left or longer spicule. The position of the first pair of papillae in this group is rather variable; it may be a little posterior to the anus, or at its level or even slightly anterior to it. There are 7 to 8 pairs of *lateral* papillae which extend from the anal region to a short distance anterior to the anterior extremity of the left spicule. Except in the anal region the sub-ventral and lateral papillae alternate in position relatively to one another.

The left spicule is slender and measures 190–230μ in length. The right spicule is a stout, somewhat complicated organ, 45–55μ long. The accessory piece is about 15μ long.

The Larva.—Larvae teased out from the uterus of the female and also found in blood smears taken from the cut surface of the sore are identical with those described by Ihle and Ihle-Landenberg (1933) in the case of *S. dedoesi*. The morphology of these larvae from the sore tissue suggests that they have been deposited there by the female in an "immature" condition; that is to say, that they require further development before attaining the true microfilarial stage typical of other filarial worms. They are short and rather stumpy, up to 140μ long, with a knob-like head and a blunt undulating tail. (Fig. 8A.) In the same blood smears containing these undoubted larvae of *S. kaeli*, the writer found much larger forms which are possibly the suggested "mature" microfilaria of this species. They measure 180–220μ and in most cases are sheathed. The head is knob-shaped and the tail is gradually tapering. The anal pore is conspicuous and characteristic and the nerve ring is also clear. (Fig. 8C). These microfilariae, which were found in small numbers in the blood smears from the sore, were also found more scantily in blood smears from the neck of the same infected cow. It cannot be stated with certainty that they are the microfilariae of *S. kaeli*, but it is extremely probable that they are, and in Fig. 8B is seen a larva of *S. kaeli* which may be an intermediate stage between it and the "immature" larvae mentioned above. This larva was found once in a smear from sore tissue and differs from the uterine larva in that the tail was no longer undulating and was sharper. The anal pore was fairly distinct but the nerve-ring was not discernible. Further forms such as this would need to be studied in order to establish clearly the relationship between the mature microfilaria and *Stephanofilaria* larva.

Comparison of the Species of *Stephanofilaria*.

In the accompanying table the principal measurements of the three species of *Stephanofilaria* are given for comparison with those of *S. kaeli*. Morphological characters are also included in the table which indicate further reasons for regarding *S. kaeli* as a distinct species. It will be seen that *S. kaeli* differs from *S. stilesi* and *S. assamensis* in several of its measurements, notably the spicule length, the thickness of the body and in the case of *S. stilesi* only, in the total length of the body of the female. There appears to be no significant difference between *S. kaeli* and *S. dedoesi* so far as principal measurements are concerned, but *S. kaeli* differs markedly in three of its morphological characters (1) the presence

of an anus in the female (2) the post-anal ventral papillae are three pairs in number (3) numerous pre-anal papillae are present. The latter two characters also serve to distinguish it from *S. stilesi* and *S. assamensis*.

Table I.

	S. dedoesi	*S. stilesi*	*S. assamensis*	*S. kaeli*
Female.				
Length	6·1–8·5 mm.	5·64–5·8 mm.	7·0–9·5 mm.	6·9–9·94 mm.
Breadth ...	156–172μ	100–117μ	190–208μ	150–160μ
Anus	Absent	Present	Indistinct	Present
Male.				
Length	2·3–3·2 mm.	3·0–3·5 mm.	3·0–4·5 mm.	2·6–3·65 mm.
Breadth ...	70–90μ	40–50μ	108–126μ	80–100μ
Left spicule ...	226–230μ	276μ	150–180μ	190–230μ
Tail	22–32μ	*18μ	25–30μ	25–35μ
Post-anal ventral papillae ...	2 pairs	2–3 pairs	2 pairs	3 pairs
Pre-anal papillae	—	6 pairs	—	13–15 pairs

* Estimated from Chitwood's text-figure.

The possibility that *S. kaeli* may be the same species as *S. dedoesi* cannot be overlooked, in view of their similarity in dimensions and the proximity of their geographical distribution. Against this view are the morphological differences indicated above, together with the fact that these two species appear to be parasites of markedly different locations in the host animal. It would appear that in the present genus, as in most genera of nematodes, morphological characters should be the essential criteria of specific diagnosis. The number and arrangement of caudal papillae in the male are recognised as providing some of the most reliable of such characters and in *Stephanofilaria* they will probably prove to be of great importance. Owing to the small size of the males of this genus, the papillae are correspondingly minute and are admittedly difficult to discern, but this does not detract from their value as specific characters. A more detailed consideration of the caudal papillae of the male would therefore seem desirable, especially in view of the possibility of further examples of this genus being found in other countries and other hosts. Similarly, the presence or absence of the anus in the female deserves detailed attention. However, until the range of variability of these characters is more accurately known, the writer has no alternative to erecting *S. kaeli* as a new species.

Conclusions.

The problem of the control or eradication of Stephanofilariasis in cattle might be approached from two aspects, *i.e.*, that of (1) treatment or of (2) prevention.

(1) Little is known concerning the value of anthelmintics in removing or killing helminth parasites which inhabit the tissues rather than the intestinal tract of the host. The location in which *Stephanofilaria* is found, namely, in the epidermis, and therefore very near the external surface of the lesion, would appear, however, to make it vulnerable ; and the possibility of killing the adult worms *in situ* by local application or injection of an anthelminthic suggests itself. Experimental work on these lines might lead to useful conclusions. It has been shown that " creeping eruption," caused by the migration of the larvae of *Ancylostoma braziliense* in human epidermis can be successfully treated by local freezing with ethyl chloride or carbon dioxide snow, which kills the migrating larvae. Possibly a similar treatment of Stephanofilariasis lesions might be used successfully by killing the adult worms.

(2) Preventive methods would be dependent on a thorough knowledge of the life-cycle of the worm. It may be concluded beyond doubt, from the zoological affinities of *Stephanofilaria*, that it employs an insect for its larval development and transmission. Probably this will be found to be a blood-sucking insect or perhaps some insect (e.g. Muscidae) which feeds on sore exudations or on blood from bites of other insects. Bubberman & Kranefeld (1933) have already suggested that the disease is transmitted by biting insects and have carried out experiments with *Tabanus rubidus*, *Stomoxys calcitrans* and *Lyperosia exigua*, but with negative results. The number of different species of insects which bite cattle is very great, however, and to approach the problem of the vector of *Stephanofilaria* without some indication or clue as to what species of insects are most likely to be implicated, would be a large task depending purely on chance for its success. In the writer's opinion, a survey of the incidence of Stephanofilariasis in a given area, should first be made. There is some evidence that in certain localities cattle are free from " Filarial Sore." A comparison of the cattle-biting insect fauna of such a locality with that of an infected locality should provide useful information on the basis of which experimental work with insects could be profitably initiated.

Acknowledgments.

The writer wishes to acknowledge his indebtedness to Mr. R. Macgregor, Veterinary Surgeon, Kuala Lumpur, for his help and interest in the investigation; to Mr. M. V. Azariah, Veterinary Inspector, for useful technical assistance and to Mr. E. P. Hodgkin, Institute for Medical Research, for the two photographs of "Filarial Sore" reproduced herewith. The preliminary examination of the material was carried out at the Institute for Medical Research, Kuala Lumpur, where, through the courtesy of the Director, Dr. A. Neave Kingsbury, the writer had been stationed during 1936–37.

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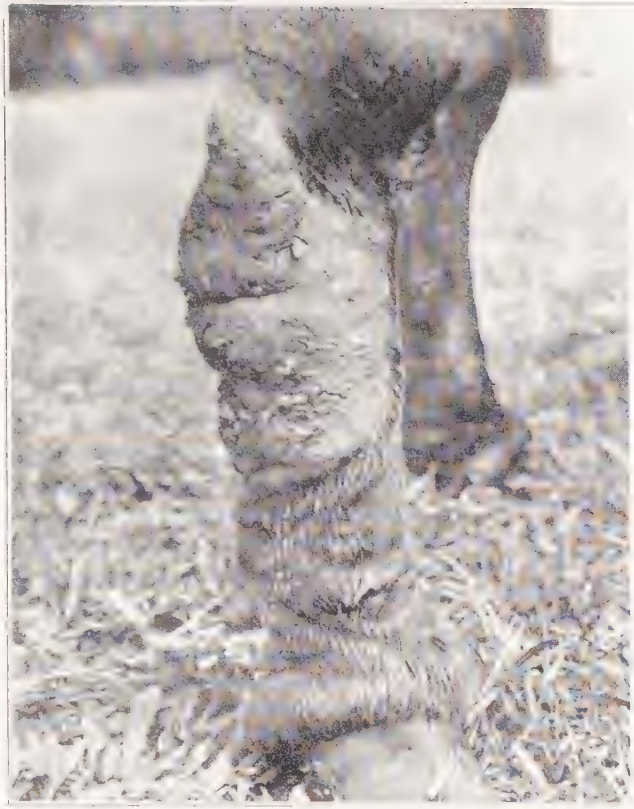
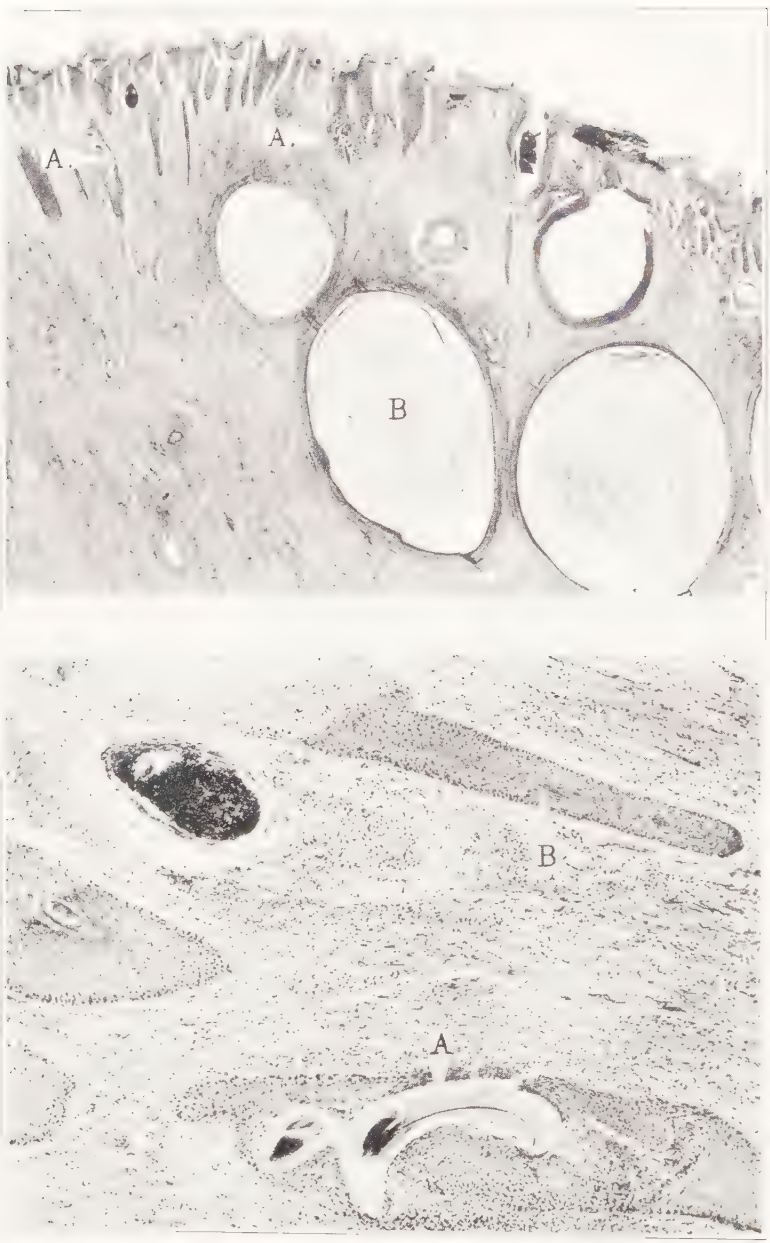


Fig. 1 — Palmar Sore — elephantoid type, in a 9 year old cow.

Fig. 2 — Palmar Sore — in a [illegible] ox.



Top—Fig. 1. (× 13.)
Bottom—Fig. 2. (× 85.)

Section of elephantoid type of "Filarial Sore."

Fig. 1. *A*. Adult worms in epithelium. *B*. Occlusion cyst.
Fig. 2. *A*. Adult worm. *B*. Epithelial process.

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The Stem Eelworm (*Anguillulina dipsaci*): Attacks on Sugar Beet.

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While examining diseased sugar beet roots at the Brigg Factory of the British Sugar Corporation, Ltd., in 1936, several roots were found which were affected by the eelworm *Anguillulina dipsaci* (Kühn). This identification was kindly confirmed by Dr. T. Goodey.

Roots which had been attacked had a raised canker round the crown. The cankered tissue was dark brown in colour and of a powdery texture, and when bits were placed in water for microscopic examination the tissues easily disintegrated. The advancing edge of the canker showed ingressing lines of discoloured tissue, often leaving islets of white tissue, and in this way was easily distinguished by macroscopic examination from the uniform advancing edge of most fungal rots. When the roots were examined in radial and longitudinal section the breakdown of the tissues was most pronounced in the intervascular areas; the vascular tissues remained white at first, but later became discoloured and disintegrated. When the vascular tissue had been attacked, the eelworm could sometimes be detected in slightly discoloured vascular tissue further down the beet longitudinally, than it appeared to have advanced in the intervascular tissue.

The attack was invariably situated round the crown of the root in the early stages and was never seen to extend more than about one-third of the way down the root on the exterior. In some cases it could be distinctly seen that the attack had taken place at the lenticels, which are normally developed round the top of the root, especially under wet soil conditions. Generally the whole surface of the crown was cankered, leaving no indication of where the attack began. The canker varied from being quite shallow to extending throughout the upper portion of the root.

Quite frequently secondary rots had begun at the canker and were spreading into the healthy tissues and it was found by isolations from the

advancing edge of the rot that *Phoma Betae* was most frequently the cause. Other fungi were sometimes isolated, *e.g.*, *Fusarium* spp. which are also parasitic on wounded beet tissues. These rots had not the characteristic powdery appearance of the canker produced by the eelworm and as *A. dipsaci* was invariably observed at the junction of the cankered and healthy tissues where there was the irregular advancing edge, there was no doubt that the eelworm was the primary cause of the canker and was attacking the living beet tissues.

Out of about 400 diseased roots collected at the Brigg Factory from representative samples of the loads sent in by growers from all over the north of Lincolnshire, 10 per cent. of the diseased roots were found to be affected with the eelworm. These were traced back to 26 growers' fields. Nine of these were situated on the Trentside Warp soil, five in the Isle of Axholme, two on the Wolds, two on light sandy soils at Scotter and Worksop, two on Carr soils and six on loam soils situated near Gainsborough, Misterton and Goxhill. With the exception of the Wold fields they were all situated on low-lying land near the Trent and Humber. Occasional affected roots were also seen at the Bardney Factory which draws beet from the south of the county, but a systematic examination was not conducted there.

When the fields yielding the affected roots were visited nearly all the crops had been lifted and no diseased beets were found growing in the fields. However all the growers agreed that the canker was seen on the roots as they were lifted. Occasional tops were collected which were known to have come from infected roots as the canker and eelworms were observed in the crown, but no distortion of the tissues or other lesions were found on the leaves or stems. In most cases all the older leaves had decayed as they had lain on the ground for some time.

When the heaps of topped beet taken from the fields were examined it was found that in some parts of the heaps 50 per cent. of the roots were infected, but in other parts no infected roots were seen. This suggested that the attacks had occurred in localised areas of the field, and this was supported by some growers' reports.

The largest roots were often the worst affected and there was no indication of the roots having been stunted by the attack of the eelworm. From the appearance of the roots the attack must have come in a late part of the season when the roots were well developed. From the commercial

point of view the loss was probably only the amount of tissues decayed, which in a few cases amounted to a considerable part of the root but usually it was not very much.

The crop rotation of some of the affected fields was traced for several years. In one badly affected field there had not been beet for four years, the intervening crops being wheat, oats and potatoes. In another case is was not known that beet had been grown in the field before, the last three crops being barley, peas and swedes. The heap of beet from one Trentside field showed 10 per cent. of the roots affected, but neither the growing plants nor the heaps of lifted roots from an adjacent field which had had a similar alternate root cereal cropping showed any sign of attack. These observations suggest that the development of a beet attacking race of *A. dipsaci* is not necessarily the result of too frequent cropping with sugar beet.

The nature of the attack on sugar beet is substantially the same as that recorded on mangolds in Herefordshire in 1927. (Goodey, 1929). In this account of the attack on mangolds, all the earlier continental records are cited.

The writers are much indebted to Mr. J. McCloy, Agriculturist of the Beet Sugar Factory at Brigg, without whose help it would have been impossible to trace the beets back to the fields upon which they were grown.

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New Names in Volume XV.

NEW GENERA.

NEW SPECIES.

Errata.

Pages 145–152, for "TOXACARA" read "TOXOCARA."

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